CHARLES UNIVERSITY IN PRAGUE

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

Department of Inorganic and Organic Chemistry

UNIVERSITY OF LJUBLJANA

FACULTY OF PHARMACY

Department of Medicinal Chemistry

DIPLOMA THESIS

Synthesis of benzothiazole derivatives as potential DNA Gyrase-B inhibitors

DIPLOMOVÁ PRÁCE

Syntéza derivátů benzothiazolu jako potenciálních inhibitorů DNA gyrázy-B

Supervisors: Assoc. prof. Dr. Janez Ilaš

PharmDr. Marcel Špulák, Ph.D.

2016

Iva Gottsteinová

Acknowledgements

I express my deep sense of thanks and gratitude to my mentor, assoc. prof. dr. Janez Ilaš from Faculty of Pharmacy, University of Ljubljana, especially for his strong leadership, endless patience, and for his valuable advices. I would like to thank teachers and other students of Department of Medicinal Chemistry for great working environment.

I am grateful to PharmDr. Marcel Špulák, Ph.D. for his guidance and support from the very beginning, as well as to all members of professor Pour's research group.

Plagiarism statement

I, Iva Gottsteinová, declare that this diploma thesis is my own work. Literature and other sources used in thesis are stated in list of reference and cited properly. This thesis was not used to acquire any other degree.

Hradec Králové Iva Gottsteinová

Abstract

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Inorganic and Organic Chemistry

University of Ljubljana
Faculty of Pharmacy
Department of Medicinal Chemistry

Candidate: Iva Gottsteinová
Supervisors: assoc. prof. Dr. Janez Ilaš, PharmDr. Marcel Špulák, Ph.D.
Title of thesis: Synthesis of benzothiazole derivatives as potential DNA Gyrase-B inhibitors

Aim of this diploma thesis was the synthesis of benzothialoze derivatives as potential inhibitors of Gyrase-B. First suggested structures were designed as analogues of alkaloid oroidin gained from marine sponge *Angelas oroides*. Successful series with high inhibitory activity were derivatives of 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole. To increase inhibitory activity, series of benzothiazole derivatives were designed. 6-nitrobenzo[*d*]thiazole-2-amine was used as starting compound and substituted in position 2 and 6 for different substitution.

In second part, this diploma thesis is focused on preparation of 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate in large quantity as a key substituent for further reactions.

Abstrakt

Univerzita Karlova v Praze
Farmaceutická fakulta v Hradci Králové
Katedra anorganické a organické chemie

University of Ljubljana
Faculty of Pharmacy
Department of Medicinal Chemistry

Kandidát: Iva Gottsteinová
Školitelé: assoc. prof. Dr. Janez Ilaš, PharmDr. Marcel Špulák, Ph.D.
Název diplomové práce: Syntéza derivátů benzothiazolu jako potenciálních inhibitorů DNA gyrázy-B

Cílem této práce byla syntéza derivátů benzothiazolu jako potenciálních inhibitorů gyrázy-B. První struktury byly navrženy jako analoga alkaloidu oroidinu získaného z mořské houby *Angelas oroides*. Úspěšnou sérií látek s vysokou inhibiční aktivitou byly deriváty 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazolu. Série derivátů benzothiazolu byla navržena, tak aby došlo ke zvýšení inhibiční aktivity. 6-nitrobenzo[*d*]thiazol-2-amin, který sloužil jako výchozí látka, byl substituován v poloze 2 a 6 různými substituenty.

V druhé části se tato diplomová práce soustředila na přípravu 3,4-dichloro-5-methyl-1*H*-pyrrol-2-carboxylátu ve velkém množství. Tato sloučenina byla využita jako substituent v dalších reakcích.

Table of Contents

1 Introduction

Antibiotics are antimicrobial drugs used in the treatment and the prevention of bacterial infections. They can possess fungicidal or antiprotozoal activity as well. Generally, they are not effective against viruses. The mechanism of action is either bacteriostatic, causing the inhibition of the growth of cells, or bactericide, which results in the death of the bacteria. Antibiotics could be divided by their origin to natural, semi-synthetic or synthetic.

1.1 History of antibiotics

People are accompanied with bacterial infections through the whole history from ancient civilizations up to this day. In ancient Egypt, Rome, Greece and China, people considered illness as punishment of gods. The herbs and other natural products with antibiotic effect were usually used to treat bacterial infections. Thus, antibacterial substances were discovered by method of trial and error.

The turning point came along when Antoine van Leeuwenhoek (1632–1723), sometimes called "the father of microbiology", designed microscope. He used it for description of seeds, nerves, muscle fibres, insect eyes, mite reproduction etc. However, the most important discovery was the revelation of small living creatures – "animalcules" in 1676.[1], [2]

In the second half of 19th century, Robert Koch (1843–1910) and Louis Pasteur (1822–1895) continued in work of Antoine van Leeuwenhoek albeit with different aim of work. Robert Koch was a physician interested in microbial causes of human diseases trying to protect human health by better hygiene. By contrast, Louis Pasteur was a chemist who worked to protect population through immunization. Because of their different general view of diseases and best possible way of improving the public health, Koch-Pasteur controversy started. In the upshot, their rivalry had both positive and negative impact.[3]

Modern era of the fields of hematology, immunology, pharmacology and chemotherapy, began when German biochemist and bacteriologist Paul Ehrlich (1854–1915) started his study on the antibacterial effect of dyes. Ehrlich described various leukocytes and established basic principles of translational medicine. In 1897, Ehrlich laid foundations of receptor-ligand concept, being formulated by his side-chain theory describing the existence of membrane related structures which can interact with extracellular molecules. After formulation of this theory, Ehrlich continued his work by developing target-specific drugs that might kill bacteria

specifically. The most famous example was his discovery of arsphenamine (Salvarsan®), the synthetic drug introduced in 1909 (Figure 1).[4]

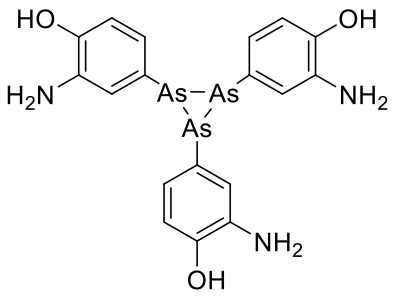


Figure 1. Structure of arsphenamine[5]

Before the discovery of penicillin by Alexander Fleming (1881–1955) in 1929, several scientists found out that certain types of moulds were able to inhibit bacterial growth. Fleming realized the great potential of penicillin, but he was not able to resolve the persisting problems with the reproduction of *Penicillium fungi*, purification and stability of the active substance. In 1940, Oxford team led by Howard Florey (1898–1968) and Ernest Chain (1906–1979) described reliable method of the purification of penicillin, which resulted, in 1945, the publication enabling the production and the distribution of penicillin.[1], [6]

The period between 1950s and 1970s is considered as "The Golden Age" of antibiotic discovery. Initially, the main sources of new antibacterial agents appeared to be naturally occurring microorganisms (e.g. *Penicillium*, *Cephalosporium*, *Streptomyces*). The novel antibiotics representing different structures and the mechanism of action were described and put into practice (Figure 2). As the number of new antibiotics increased, the first observations of the antibiotic resistance started to appear. Thus, the natural sources of antibacterial compounds became insufficient resulting in the necessity of the chemical modifications of existing drugs.[1], [6]

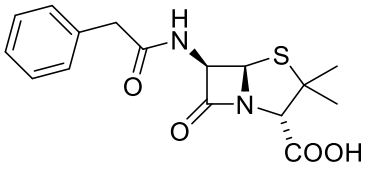


Figure 2. Structure of benzylpenicillin (G-penicillin)[27]

Nowadays, many of drugs being discovered in the last century are still used in the treatment of infectious diseases. Their role in the modern medicine is irreplaceable and indispensable. Even-present problem remains to be antibacterial resistance. Overusing and misuse leads to multidrug-resistant bacterial infections which are responsible for the high mortality rate in Europe and all around the world. Although the majority of bacterial infections were placed under control, the researchers are facing an task induced by the rising number of multidrug-resistant bacteria permanently increasing.[6]

This diploma thesis focused on synthesis of new benzothiazole derivatives as potential DNA Gyrase-B inhibitors. Specific mechanism of action of DNA gyrase inhibitors led to discovery of many potential structures which are or could be used as antibacterial or anticancer drugs. Not only increasing resistance and a number of side effects, but also high antibacterial and anticancer potential of DNA gyrase inhibitors attract attention of many research groups all over the world.

1.2 DNA

Deoxyribonucleic acid (DNA) carries the genetic information for the synthesis of specific proteins. In 1953, American biologist James Watson (born 1928) and British physicist Francis Crick (1916-2004) presented their model of double helix. Revelation of DNA structure led to understanding of transcription, translation and cell replication.[7], [9]

DNA strand is composed of the repeating monomeric units called nucleotides. Nucleotide consists of five-membered sugar – *D*-pentose (*D*-2-deoxyribose), phosphate and purine (adenine, guanine) or pyrimidine (cytosine, thymine) base. The backbone of DNA strand is made by covalent phosphodiester bond between 3′-hydroxyl group of one sugar and 5′-hydroxyl group of the adjacent one. Nucleic base is bound to position 1′ of 2-deoxyribose by *N*-glycosidic linkage. Chemical composition is considered as primary structure of DNA (Figure 3).[7], [8], [9]

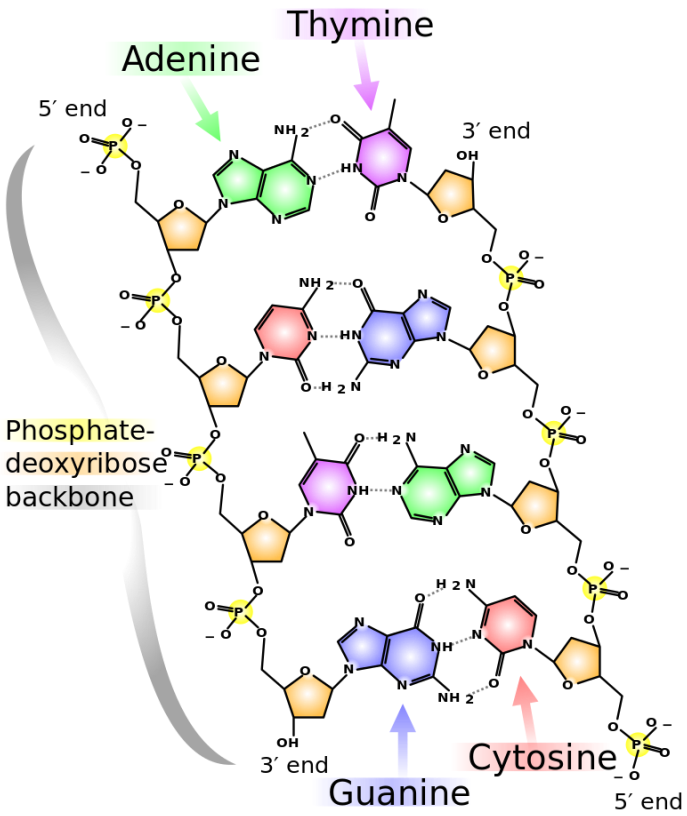


Figure 3. The secondary DNA structure[10]

The molecule of DNA consists of two polynucleotide strands twisted in the shape of double helix that is considered as secondary structure of DNA (Figure 3). Firstly, strands of DNA are antiparallel, i.e. one strand is in direction 3′-5′, the second in direction 5′-3′. Numbers 3′and 5′ correspond to carbons of 2-deoxyribonucleose and determinate terminal groups of strands. 3′End has terminal hydroxyl group, 5′end has terminal phosphate group. Secondly, nucleotides of DNA chains contain different bases that have ability to create hydrogen bonds between them. Adenine is always paired with thymine by two hydrogen bonds, while guanine is paired with cytosine by three hydrogen bonds. As the result, the every base pair is formed by one purine and one pyrimidine base bound to each other by hydrogen bound across double helix. Antiparallel strands and base pairs influence the final secondary structure. The most common form of double helix is right-handed double helix which occurs in two different conformations – B-form (the most frequent) and A-form. Rarely, some repeated nucleotide sequences of purines and pyrimidines have form of left-handed double helix called Z-form.[7], [8]

Prokaryotic cells generally possess one circular chromosome, called nucleoid, composed of double-stranded DNA molecule and proteins considered as nucleoid-associated

proteins (NAPs).[11], [12] Highly condensed plectonemically supercoiled DNA is not stored in nucleus but usually freely floats in cytoplasm.[11] Occasionally, DNA is attached to the cell membrane.[7] The torsional tension of supercoiled DNA in bacteria serves as reservoir of free energy used during important processes as transcription or replication.[11] Transcription is also influenced by NAPs in both manners – positive or negative.[12]

Plasmids are DNA molecules that exist independently on chromosome. The structure is created by supercoiled double-stranded DNA molecule, usually circular. The length of plasmids is diverse from hundreds to thousands of base pairs. Plasmids are not vitally important, however, they can provide new abilities to prokaryotic cells as antibiotic resistance or special metabolic properties. Revelation of plasmids and their function led to the development of genetic engineering, especially on the field of the creation of new artificial plasmid vectors.[7], [13]

Eukaryotic cell contains one linear double-stranded DNA placed in nucleus. On the basics of DNA length, the structure of eukaryotic chromosome has to be composed by several substructure units. Fundamental structure, nucleosome, is constituted by histone octamer and DNA strand. Histones are basic proteins with positive charge that enable to create relatively strong bonds to negatively charged phosphate groups of DNA. Histones are divided into five groups, described as H1, H2A, H2B, H3 and H4. Histone octamer contains two of each kind - H2A, H2B, H3 and H4. One hundred and forty-six base pair long section of DNA chain is twined around each histone octamer approximately two and half times, followed by sixty base pair long connecting part.[7], [8] Ten nanometer long chromatin filaments composed of nucleosomes, sometimes described as “beads-on-string” structure, condense and create thirty nanometer chromatin fiber described as classical solenoid (Figure 4).[15] The formation is initiated by histone H1 which connects the histone octamer of one nucleosome to the first base pairs of connection part of adjacent one.[7], [8] As condensation proceeds, loops of solenoids accumulate on chromatin fiber and form chromomeres. Then, as the filaments become shorter and thicker, chromatin forms the final structure – chromosome.[15] The level of the chromatin condensation varies in different areas of chromosome. Euchromatin is less condensed and contains actively expressed genes, on the other hand, heterochromatin is highly condensed and does not contain actively expressed genes.[14] Non-histone proteins, usually acidic, playing an important role during condensation. They are attached to chromatin, thus they maintain the different spatial arrangements and conformations.[7]

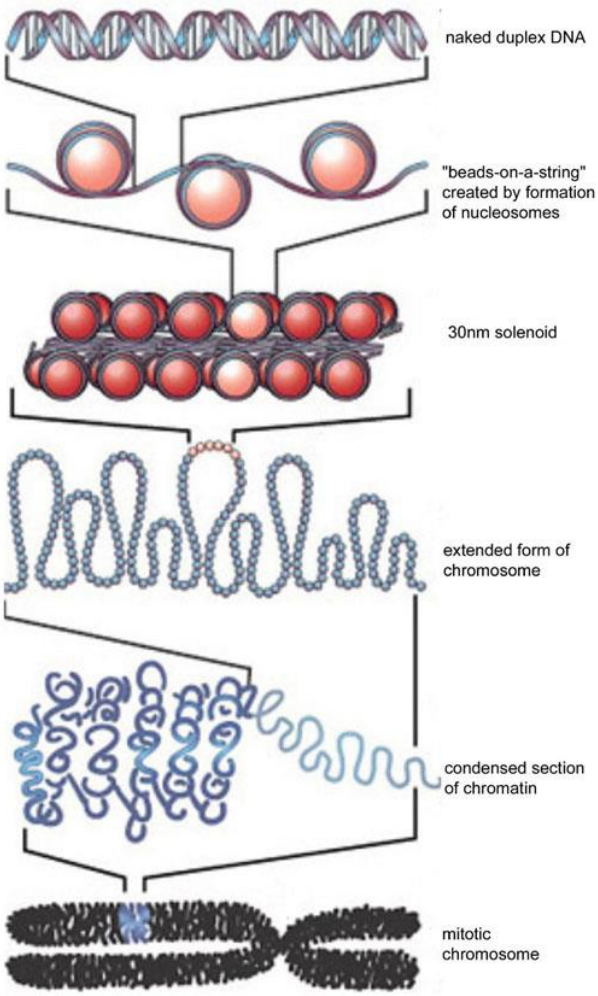


Figure 4. Scheme of the different levels of DNA condensation[16]

1.2.1 DNA topology

The topology of DNA is defined by the three-dimensional structure of two antiparallel polynucleotide strands that are multiply intertwined in the way they are forming a right-handed double helix.[17], [18] The semiconservative replication model requires untangled double helix, however untangling depends on the free rotation of DNA ends.[17], [19] Untangling the two DNA chains is topologically impossible because, naturally, free end rotation is restricted or forbidden. DNA segment with absolutely limited movement is called topological domain. For example, bacteria contain circular DNA where both ends of DNA chains are covalently closed. However, topological domains appear in eukaryotic cells as well, for example chromosomal DNA loops, DNAs attached to the membrane or protein aggregates.[17] Topological homeostasis and correct DNA functions are secured by enzymes called DNA topoisomerases. DNA topoisomerases influence DNA packing, condensation, transcription, chromosome segregation, and gene

expression.[19] During the strand separation in topological domain, topoisomerases can release torsional tension by transient breaks in DNA backbone.[17], [19] DNA topology includes supercoiling, knots and catenanes.[19]

1.2.1.1 Supercoiling

Supercoiling (Figure 5), compensatory mechanism of covalently closed DNA which is permanently under torsion tension, is usually described by three numbers – linking number, twist number and writhe number.

Linking number (Lk) represents number of links between two complementary single strands of covalently closed circular DNA. Lk is always positive or negative integer, and topologically invariant, i.e. that it cannot be changed without temporary breaks of one or both DNA strands.[17], [18], [19]

Twist number (Tw) "is the total number of helical turns in circular DNA under given conditions".[17]

Writhe number (Wr) describes how many times the double helix is turned around itself in superhelix.[18]

For right-handed helix, Lk and Tw are defined as positive numbers, meanwhile Wr has negative value.[19]

Equation

$$Lk = Tw + Wr$$

shows relation among Lk, Tw and Wr. In case that DNA strands are locally separated, Tw decreases and torsional tension increases. Torsional stress has negative impact to DNA molecule and thus it is necessary to compensate it. However, Lk value cannot be changed, and thus compensatory mechanism is based on writhes. Decreasing Tw in double helix will be balanced by writhes in opposite direction.[19]

Right-handed DNA helix without torsional stress and with approximately 10.5 base pairs per turn is considered as "relaxed" B-form DNA.[17], [18] In this case, relaxed linking number (Lk_0) equals Tw.[17], [19] However, relaxed DNA represents theoretical and nonexistent condition in nature. The difference between real linking number (Lk) of molecule and theoretical linking number of completely relaxed DNA (Lk_0) is called ΔLk (linking

difference).[18] If ΔLk differs from zero, supercoiling appears. Winding DNA around its axis in the same direction as the helix, corresponds to positive value of Lk and generates positive supercoiling, whereas winding in opposite direction leads to negative value of Lk and introduced negative supercoiling.[17], [20]

$$\Delta Lk = Lk - Lk_0 \quad (2)$$

Generally, supercoiling describes the shape of the DNA. Supercoiled DNA occurs in two configurations – plectonemic or solenoidal. Plectonemic supercoiling is characteristic DNA conformation in prokaryotes. In eukaryotes, both conformations occur, however more frequent solenoidal supercoiling provides better DNA organization achieved by twisting of helix around histones.[17] Negative supercoiling usually can promote many DNA processes, while positive supercoiling can inhibit them.[19]

1.2.1.2 Knots and catenanes

Knots and catenanes (Figure 5) are other topologically invariant features for covalently closed circular DNAs. Thus, their attributes cannot be modified by conformational modifications, only strand breaks of DNA produced by topoisomerases can change final conformation. Knots are formed during cyclization of long DNA molecules probably as side products of genetic processes. Knots occur in various forms, however they are detected in living cells rarely. Catenanes are defined as two or more circular DNA molecules linked together during process of cyclization. In contrast to knots, catenanes are formed during DNA replication and detected in living cells ordinarily.[17]

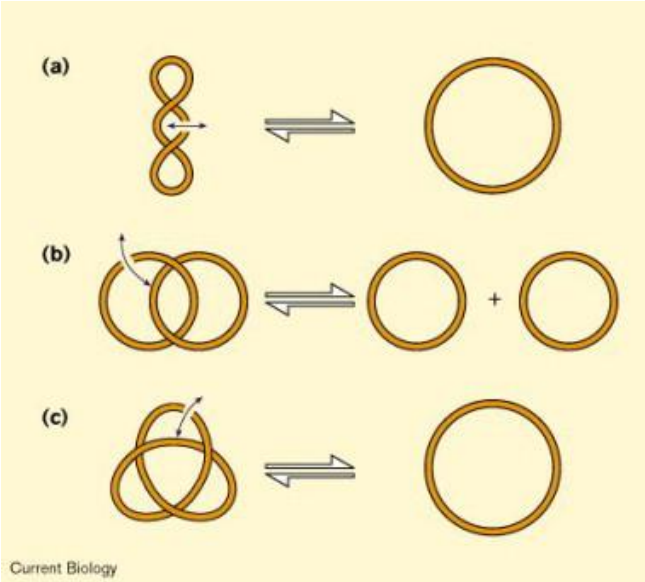


Figure 5. Reactions caused by topoisomerases: (a) supercoiling/relaxation; (b) catenation/decatenation; (c) knotting/uknotting[21]

1.3 Topoisomerases

Topoisomerases are enzymes vitally necessary for all living organisms. Topoisomerases regulate the topological state of the DNA by creation of transient breaks in one (type I subfamily) or in both strands (type II subfamily) of double helix.[18], [22] With a few exceptions, topoisomerases relax only negative supercoils.[23] Unwinding and bending of DNA during changing DNA topology can promote catenation and knotting of circular DNAs, or disentanglement of twined linear DNA strands in eukaryotic cells. [23], [24]

Topoisomerases have irreplaceable role during transcription, recombination or replication. Differences between bacterial and human topoisomerases became crucial for synthesis of new topoisomerase-targeted drugs, called inhibitors of topoisomerases. Mechanism of action is based on creation of covalent bond between targeted topoisomerase and drug agent. Antibiotics affect bacterial topoisomerases, whereas anticancer chemotherapeutics inhibit human topoisomerases.[19], [23]

1.3.1 Topoisomerase I

Topoisomerases I cleave temporary single-stranded breaks in the double helix, it leads to the releasing of one DNA strand end that bonds to the tyrosine residue by temporary

phosphodiester bond. Subtype IA binds the free end to 5′-terminal phosphate group (type I-5′) and enables passage of intact strand through the cleavage, whereas subtype IB attaches the free end to 3′-terminal phosphate group (type I-3′) and so another end of strand can rotate around the intact one.[18], [23], [24] Topoisomerases change Tw by removing one turn of supercoiling, thereby they decrease torsional helical stress of supercoiled molecule.[18]

Characteristics for subtype IA:

- Monomeric (except for *Methanopyrus kandleri* reverse gyrase)
- One free end of DNA strand bonds to 5′-terminal phosphate group
- DNA relaxation requires Mg^{2+}
- Relaxation of negative supercoils
- Incomplete relaxation
- Alteration of Lk in step of one
- Catalysis of knotting, unknotting and interlinking of single-strand DNA and in addition catenation and decatenation of double-strand DNA

Characteristics for subtype IB:

- One free end of DNA strand is attached to 3′-terminal phosphate group
- DNA relaxation does not require Mg^{2+}
- Relaxation of both, positive and negative supercoils
- Complete relaxation[18], [23]

1.3.2 Topoisomerase II

Topoisomerases II cleave both strands of the double helix and enable passage of another double helix through transient break.[18], [23], [24] Topoisomerases II are ATP-dependent and the relaxation requires Mg^{2+}. Topoisomerases II remove one writhe, thus Lk number alters in steps of two.[18], [23] Topoisomerases II can relax negatively and positively supercoiled DNA and provoke catenation, decatenation, knotting and unknotting.[25]

Both, prokaryotic and eukaryotic topoisomerases possess DNA binding domains and ATPase domains, each domain is present twice. Eukaryotic enzymes are homodimers, the ATPase domain is placed on the *N*-terminal area of enzyme, while the active tyrosine site

is located on the *C*-terminal area.[18], [23], [25] Prokaryotic enzymes are heterotetramers. In DNA gyrase, active tyrosine site is located on the A subunit of DNA gyrase (GyrA), whereas ATPase domain is placed on the B subunit of enzyme (GyrB). Subunits A (97 kDa) and B (90 kDa) create A_2B_2 tetramer. In topoisomerase IV, subunits C (ParC) and E (ParE) are homologous to subunits A and B, respectively, and create C_2E_2 complex.[25], [26]

Mechanism of action is described as "two-gate mechanism". A segment of DNA called G segment is caught by enzyme. Segment G is cleaved and both strands are attached to 5´-terminal phosphate group on GyrA (through covalent phosphotyrosine bond). ATP binds to GyrB, conformation of enzyme changes and clamps are formed. The T segment is captured into the gate. While T segment is passing through, energy released by ATP hydrolysis induce conformation changes, clamps close and new protein gate is created on the opposite site. Segment T exits enzyme and segment G is reunited. The result is creation of two negative supercoils.[23], [26] (Figure 6)

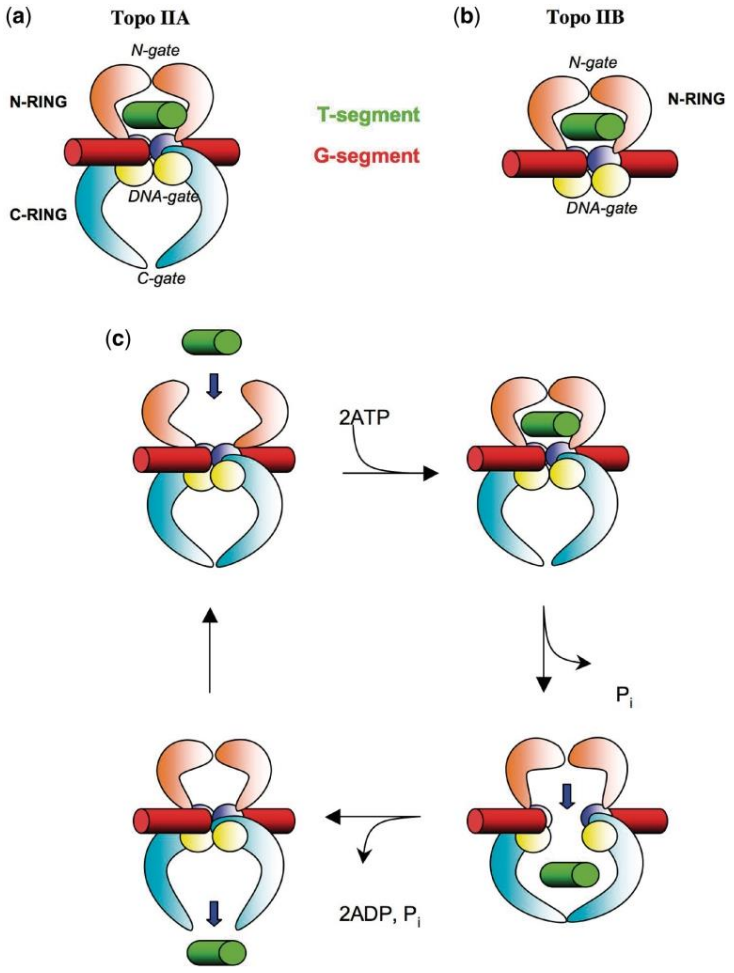


Figure 6. (a) Structure of topoisomerase IIA; (b) Structure of topoisomerase IIB; (c) Scheme of catalytic cycle of topoisomerase II: (1) binding and cleavage of G-segment binding; (2) ATP binding, conformation change and T-segment capturing; (3) DNA strand passage, G-segment relegation, ATP hydrolysis; (4) T-segment releasing, enzyme recycling[22]

DNA gyrases and topoisomerases IV belong to subfamily topoisomerase IIA. DNA gyrases are the only DNA topoisomerases that can actively underwind DNA, i.e. introduce negative supercoils. DNA gyrases are fundamental and indispensable enzymes in bacteria. Topoisomerases IV differs from DNA gyrases in structure of their *C*-terminal domain. The main function of topoisomerases IV is the relaxation of positive supercoils. While DNA gyrase is essential enzyme, topoisomerases IV are uncommon and usually, they occur with DNA gyrase together in bacteria. In this case, their roles in bacteria are overlapping.[25]

Topoisomerases differs from each other by structures and functions. Primarily, structural differences between prokaryotic and eukaryotic enzymes are interesting as potential pharmaceutical targets. Antibacterial drugs inhibit bacterial DNA gyrase or topoisomerase IV, while anticancer drugs stop cancer cells proliferation by inhibition of human topoisomerase I or II.[18] Inhibitors of topoisomerases act either as catalytic inhibitors (i.e. they inhibit ATPase activity, e.g. novobiocin), or as more efficient cleavage-complex stabilizing agents (e.g. ciprofloxacin).[26]

1.4 Inhibitors of topoisomerases as antibacterial drugs

Inhibitors of topoisomerases are antibacterial drugs that inhibit bacterial DNA gyrase or topoisomerase IV. Based on their structure and mechanism of action, these antibacterial agents are divided into two groups – quinolones and aminocoumarins.

1.4.1 Quinolones

Generally, quinolones are synthetic derivatives of 4-oxo-1,2-dihydroquinolin-2-carboxylic acid and its aza-analogues. The first synthetized molecule, nalidixic acid, was introduced to the market in 1962, and became a model for a synthesis of new antimicrobial agents. Nowadays, quinolones are divided into four generations on the ground of their structure and antibacterial spectrum.[26], [27]

Quinolones possess mainly bactericidal activity. During relaxation of supercoiled DNA, when free DNA ends are attached to the A subunit of DNA gyrase, DNA-GyrA complex is formed. This cleavage-complex can be stabilized by quinolones. The higher stability of complex, the higher effect is. Creation of cleavage-complexes leads to chromosome fragmentations and it results in cell death. Quinolones inhibits ATPase activity as well.[18], [27]

First generation (e.g. nalidixic acid) of quinolones are narrow-spectrum antibiotics, mainly effective against gram-negative bacteria. Spectrum broadening was reached by introduction of fluorine to the molecule. Therefore, the second generation (e.g. norfloxacin, ciprofloxacin, ofloxacin) has enhanced activity against gram-positive bacteria. Further modifications of the basic structure led to the synthesis of new broad-spectrum antibiotics belonging to the third (e.g. sparfloxacin) and fourth generation (e.g. moxifloxacin) (Figure 7). Nowadays, first three generations of quinolones are clinically used in the treatment of urinary,

respiratory and gastrointestinal infections, whereas quinolones of fourth generation are reserve antibiotics.[18], [26], [27], [28]

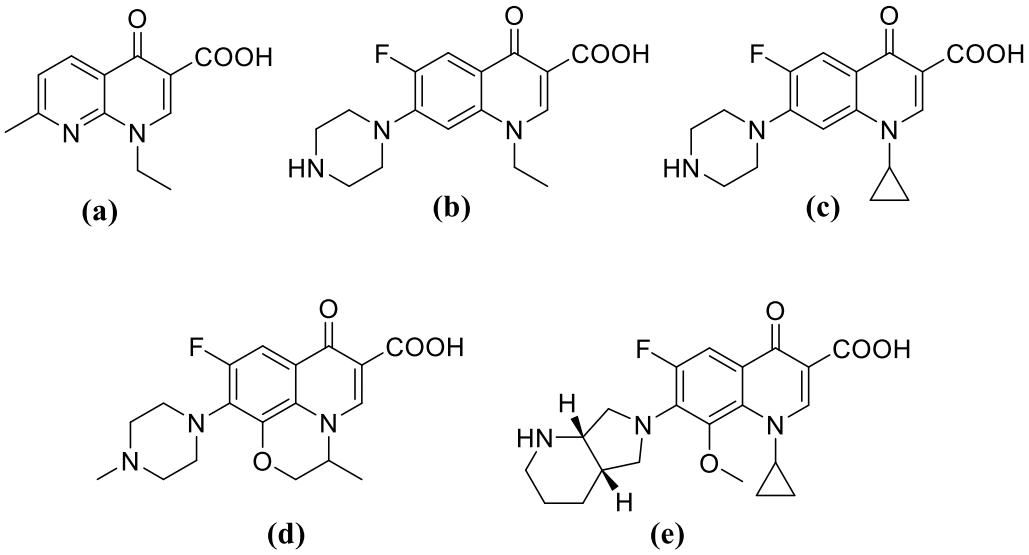


Figure 7. (a) nalidixic acid; (b) norfloxacin; (c) ciprofloxacin; (d) ofloxacin; (e) moxifloxacin[27]

Increasing bacterial resistance is worldwide problem. Resistance to quinolones is usually caused by mutations in the genes encoding topoisomerases. The others are especially lipopolysaccharide or efflux mutations. All these mutations decrease stability of the cleavage-complex.[29]

1.4.2 Aminocoumarins

Aminocoumarins are natural products isolated from *Streptomyces* genus. Their structure contains 3-amino-4,7-dihydroxycoumarin ring. Novobiocin was the first aminocoumarin introduced to the clinical use in 1964. Novobiocin, clorobiocin and coumermycin A_1 (Figure 8) differs from each other in the substitution of the central ring.[26], [30]

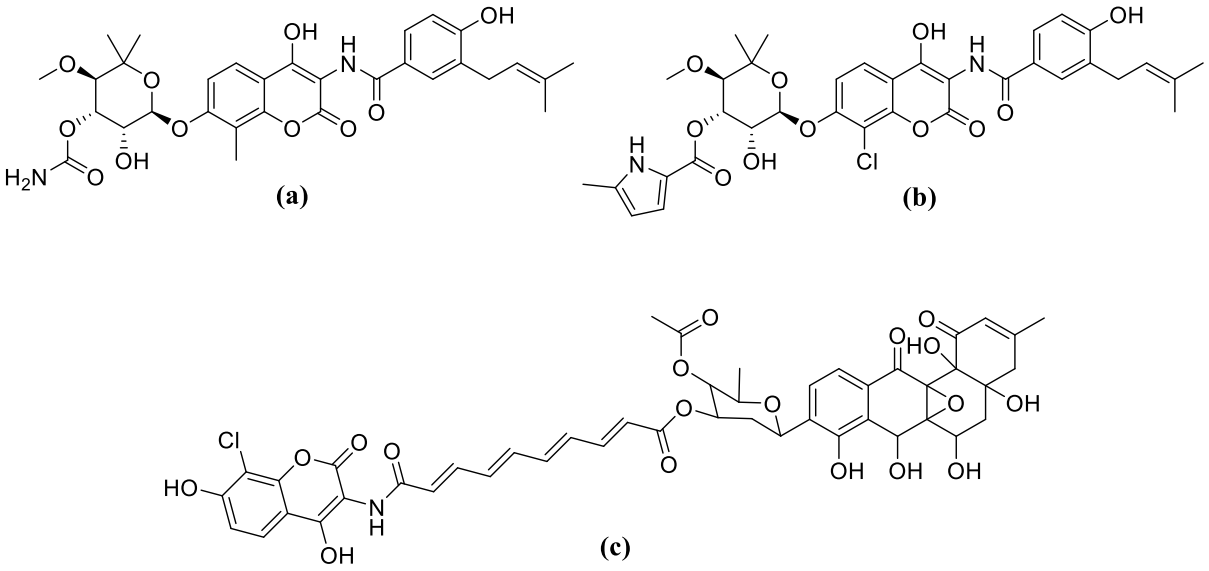


Figure 8. Structure of novobiocin (a), clorobiocin (b) and simocyclinone D8 (c) [26]

Aminocoumarins are competitive inhibitors, that inhibit ATPase activity by binding to the ATP-binding site on GyrB instead of ATP. Bacterial resistance to aminocoumarins is caused by spontaneous mutations of amino acids in the GyrB protein chain.[26]

Simocyclinones are aminocoumarin derivatives containing polyketide moiety. Simocyclinone D8 (Figure 8), effective against gram-positive bacteria, inhibits DNA gyrase and human topoisomerase II. In contrast to others aminocoumarins, simocyclinone D8 binds to the GyrA and prevents DNA binding to this subunit.[26]

Aminocoumarins are highly effective against gram-positive bacteria, however they have poor activity against gram-negative bacteria. Moreover, low solubility and high eukaryotic cytotoxicity, limits their clinical usage. Nevertheless, the difference in mechanism of action between aminocoumarins and quinolones is increasingly important as the number of quinolone-resistant bacteria grows.[26], [30]

1.5 Inhibitors of topoisomerases as anticancer drugs

Inhibitors of topoisomerases as anticancer drugs inhibit human topoisomerase I and II and decrease cell proliferation.

1.5.1 Camptothecin and its derivative

Camptothecin is basic quinoline alkaloid isolated from the bark of Chinese tree *Camptotheca acuminate*. Camptothecin inhibits human topoisomerase I by stabilization of cleavage-complex (DNA is cleaved and free ends are attached to GyrA). Because of its high toxicity and instability, camptothecin is not in the clinical usage anymore, however its structure became a model for the synthesis of new the derivatives with enhanced properties – topotecan and irinotecan. The main indication of topotecan is ovarian cancer. Irinotecan is used in the treatment of colorectal cancer, ovarian cancer and cervical cancer (Figure 9). [18], [27]

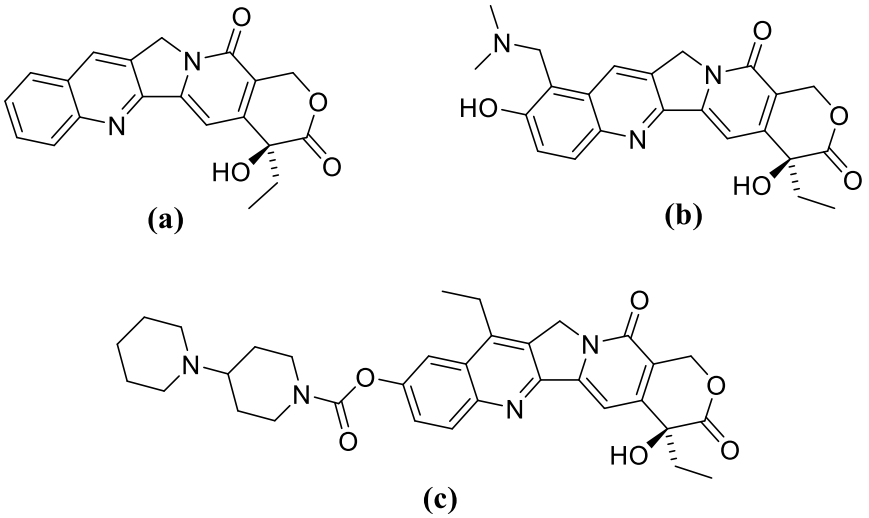


Figure 9. (a) camptothecin; (b) topotecan; (c) irinotecan[27]

1.5.2 Podophyllotoxin and its derivatives

Podophyllotoxin is non-alkaloid compound isolated from roots of *Podophyllum* species. The mechanism of action is the inhibition of human topoisomerase II. Podophyllotoxin is used in treatment of viral papilloma. Etoposide, semi-synthetic derivative of 4′-demethyl-9-epi-podophyllotoxin, is clinically used in the treatment of leukemia, lymphoma, testicular cancer and lung cancer.[18], [27]

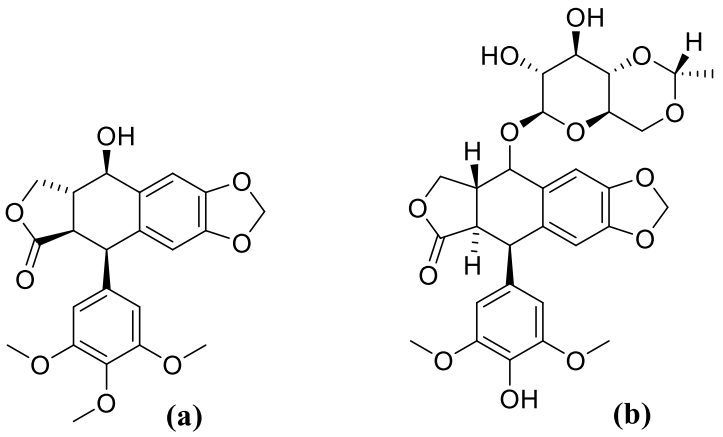


Figure 10. Structure of podophyllotoxin (a) and etoposide (b)[27]

1.6 New potential inhibitors of topoisomerases

Nowadays, the increasing bacterial resistance through all antibiotic classes becomes serious global problem, therefore it is necessary to search for the new antibacterial agents. Scientists research natural resources as a potential source of new antimicrobial compounds.

Oroidin (Figure 11), pyrrole alkaloid, was discovered and isolated from the marine sponge *Agelas oroides* in 1971. Oroidin has modest anti-protozoal and poor cytotoxic activity. However, on the ground of its simple structure, chemical modifications can be introduced easily. Oroidin, together with alkaloids hymenidin and clathrodin, can be considered as cornerstones for synthesis of new DNA gyrase inhibitors.[31], [32]

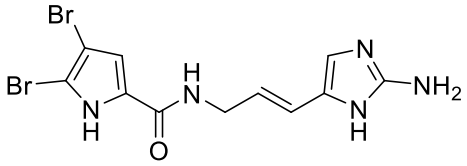


Figure 11. Structure of oroidin[31]

Structural similarities between DNA gyrase and topoisomerase IV are employed for dual targeting in most bacteria. Potential GyrB and dual targeting GyrB/ParE inhibitors were discovered, e.g. among derivatives of azaindoles, pyrrolo[2,3-*d*]pyrimidines, imidazo [1,2-*a*]pyridine ureas, pyrrolamides. However, none inhibitor was introduced to clinical usage yet.[33], [34]

New potential DNA gyrase inhibitors were derived from marine alkaloid clathrodin (Figure 12), specifically for its pyrrole amide structure. Basic pyrrole amide structure was bonded to 5,6,7,8-tetrahydroquinazoline or 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole. Inhibitory activity was tested on *E. coli* DNA gyrase. The best *in vitro* inhibitory activity of the 5,6,7,8-tetrahydroquinazoline derivatives had analogues containing 4,5-dibromo-1*H*-pyrrole. Analogously, derivatives of 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole containing 4,5-dibromo-1*H*-pyrrole had the best *in vitro* inhibitory activity. It shows that bromine atoms are important during formation of hydrophobic bonds with Asp73 of the GyrB. Replacement of 4,5-dibromo-1*H*-pyrrole by 4,5-dichloro-1*H*-pyrrole did not lead to higher inhibitory activity.[33]

Substitution of 4,5,6,7-tetrahydrobenzothiazole by new central compound containing 4-aminobenzamide group, 4-aminophenol group or 4-aminobenzene ring with basic side chain, led to synthesis of new potential DNA gyrase inhibitors. 4,5-dibromopyrrolamide derivatives possessed the highest activity.[34]

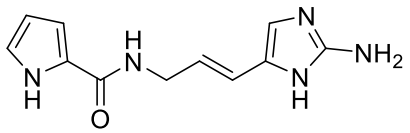


Figure 12. structure of clathrodin[33]

2 Aim of work

Firstly, the aim of my diploma thesis was the synthesis of new benzothiazole derivatives as potential DNA gyrase-B inhibitors. Structures were designed according to the previously prepared compounds possessing the best *in vitro* inhibition.[33], [34] Initial structure of 6-nitrobenzo[*d*]thiazole-2-amine was substituted in position 2 and 6 with different substituents (Figure 13).

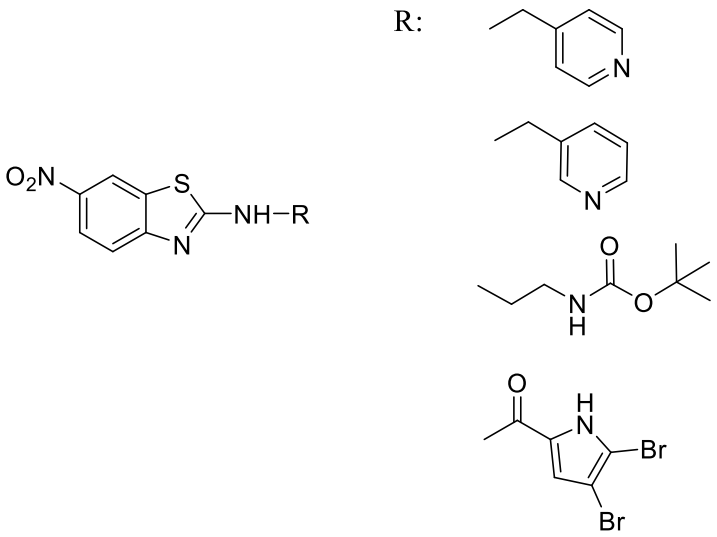


Figure 13. Structure of 6-nitrobenzo[*d*]thiazole-2-amine

Secondly, I focused on the preparation of 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate in large quantity as a substrate for the further reactions (Figure 14).

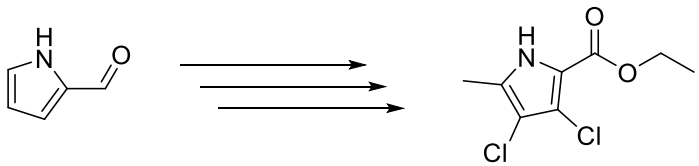


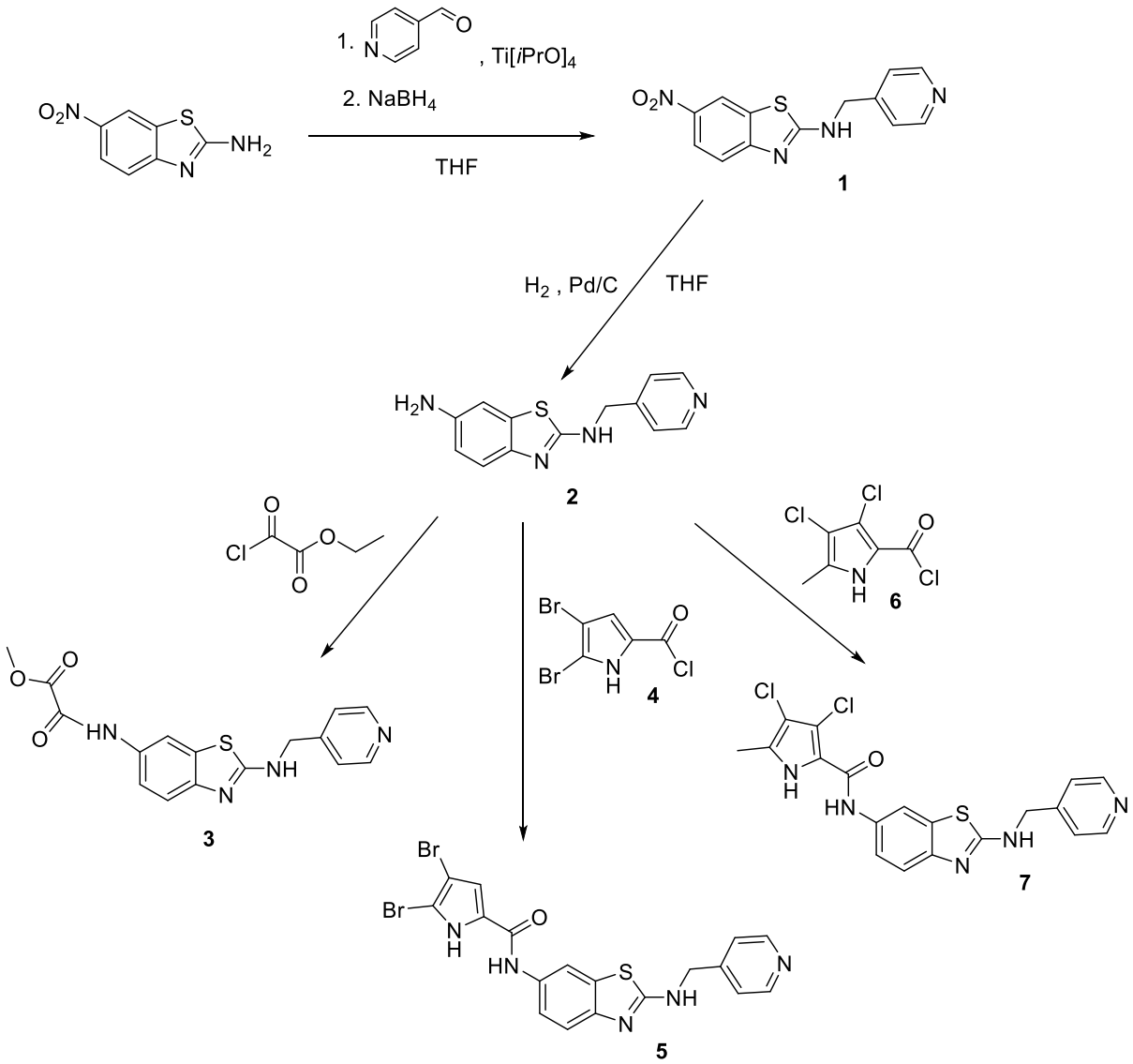
Figure 14. Synthesis of 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate

3 Results and discussion

3.1 Synthesis of benzothiazole derivatives

3.1.1 Synthesis of N^2-(pyridine-4-ylmethyl)benzo[*d*]thiazole-2,6-diamine derivatives

Synthesis of benzothiazole derivatives started with the commercial available 2-amino-6-nitrobenzothiazole. Amino group underwent the reductive amination sequence, followed by the reduction of nitro group leading to amine group and its further modification (Scheme 1).

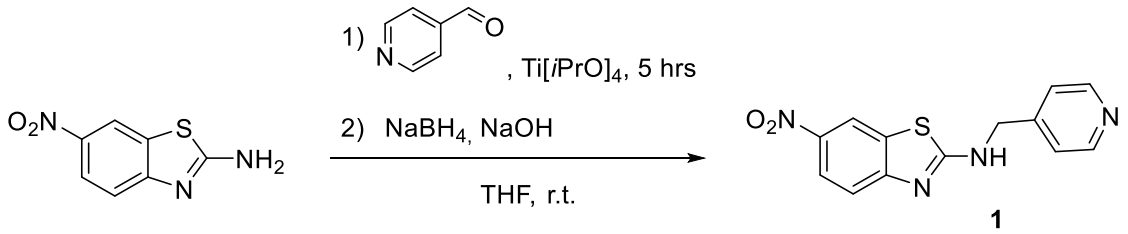


Scheme 1.

In the first step of the synthesis, 2-amino-6-nitrobenzothiazole reacted with 4-pyridinecarboxyaldehyde in the presence of titanium(IV)isopropoxide to afford 6-nitro-*N*-(pyridine-4-ylmethyl)benzo[*d*]thiazol-2-amine (**1**). After the reduction of nitro group of compound **1**, the obtained N^2-(pyridin-4-ylmethyl)benzo[*d*]thiazole-2,6-diamine (**2**) was coupled with ethyl oxalyl chloride, 4,5-dibromo-1*H*-pyrrole-2-carbonyl chloride (**4**) or 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carbonyl chloride (**6**) to yield the desired compounds **3**, **5** and **7.**

3.1.1.1 Synthesis of 6-nitro-*N*-(pyridin-4-ylmethyl)benzo[*d*]thiazol-2-amine

First step of the reaction was reductive amination[35]. 1.0 equivalent of 2-amino-6-nitrobenzothiazole was dissolved in THF and amino group reacted with 1.2 equivalents of 4-pyridinecarboxyaldehyde in the presence of 1.7 equivalents of titanium(IV) isopropoxide to afford imine which was confirmed by TLC. Subsequently, the resultant imine was reduced by 1.7 equivalents of reducing agent $NaBH_4$ and reaction was quenched by 0.4 M NaOH. To afford the pure product **1**, recrystallization from ethanol was carried out yielding 19% (Scheme 2).



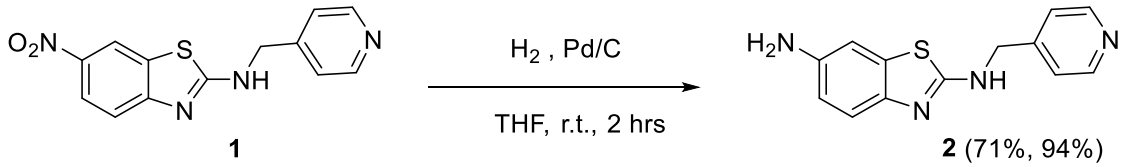
Scheme 2.

According to the synthetic plan, compound **1** was essential for the synthesis of different derivatives. Unfortunately, the yield was low and the reaction had to be repeated several times. Based on TLCs, reactions always run completely to imine, problems started to appear after quenching by sodium hydroxide when the decomposition leading to initial substrates occured. Therefore, the reaction conditions were changed – different equivalents of 4-pyridinecarboxyaldehyde and catalyst were added (Table 1); different concentration and the amount of sodium hydroxide were used; different recrystallizations were made; also freshly opened reactant were used for reaction.

Table 1.

Formula	$C_7H_5N_3O_2S$	C_6H_5NO	$Ti[iPrO]_4$	$NaBH_4$	Yield (%)
MW	195,20	107,11	284,22	37,83	-
Eq 1st reaction	1	1,2	1,7	1,6	19
Eq 2nd reaction	1	1,2	1,7	1,6	27
Eq 3rd reaction	1	2,2	1,7	1,6	0
Eq 4th reaction	1	1,2	3,4	1,6	0
Eq 5th reaction	1	1,2	1,7	1,6	Less than 1
Eq 6th reaction	1	1,2	3,4	1,8	0

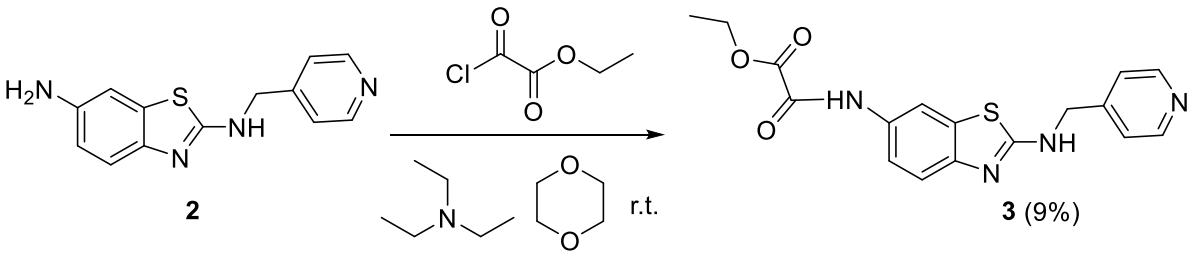
The second step of the reaction sequence was the catalytic reduction of benzothiazole nitro group, employing 50 mg of palladium on carbon as catalyst in hydrogen atmosphere in THF (Scheme 3). Compound **2** was used in the next steps without further characterization. The reaction was performed twice affording 71% and 94% yields.



Scheme 3.

3.1.1.2 Synthesis of ethyl 2-oxo-2-((2-((pyridin-4-ylmethyl)amino) benzo[*d*]thiazol-6-yl)amino)acetate

Amino group attached to carbon 6 of benzothiazole can serve as a substrate for the further modifications. In this case, amino group reacted with 1.2 equivalents of 2.0 M ethyl oxalyl chloride in presence of triethylamine, using dioxane as solvent (Scheme 4). Compound **3** was isolated with poor yield of 9%. However, based on ^{1}H NMR spectrum the purity was considered as insufficient for the biological testing. Reaction was repeated again but isolation failed.

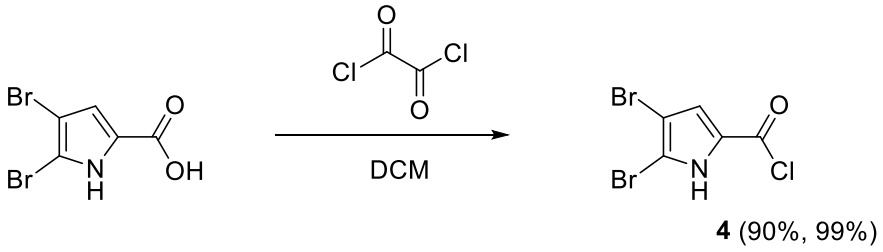


Scheme 4.

3.1.1.3 Synthesis of 4,5-dibromo-*N*-(2-((pyridin-4-ylmethyl)amino) benzo[*d*]thiazol-6-yl)-1*H*-pyrrole-2-carboxamide

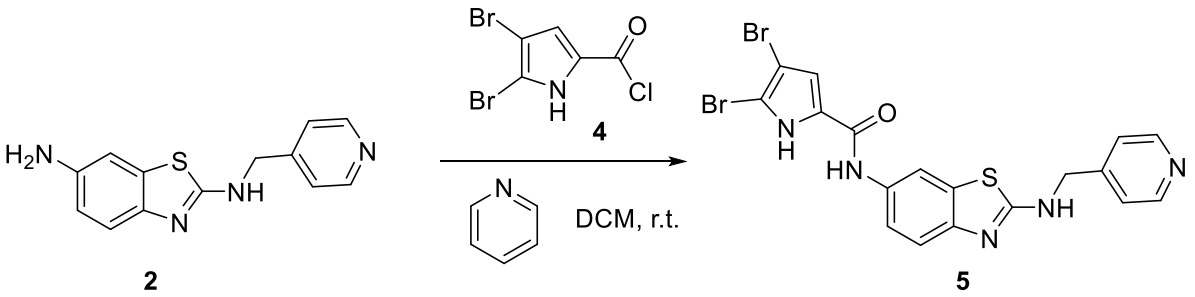
On the grounds of instability of differently substituted 1*H*-pyrrole-2-carbonyl chlorides, they had to be synthetized from the appropriate carboxylic acid precursors immediately before the further conversion.

For synthesis of the desired compound **5**, 4,5-dibromo-1*H*-pyrrole-2-carbonyl chloride (**4**) was prepared freshly from 4,5-dibromo-1*H*-pyrrole-2-carboxylic acid by reaction with 5.0 equivalents of 2.0 M ethyl oxalyl chloride in DCM (Scheme 5). Yield was 90%. Compound **4** was used in next step of reaction without further characterization. Synthesis was repeated employing the same conditions affording chloride **4** in 99% yield.



Scheme 5.

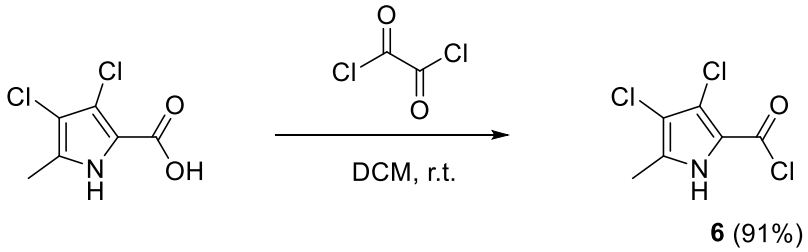
Starting aminobenzothiazole **2** underwent the reaction with 1.1 equivalents of chloride **4** in the presence of pyridine in DCM to afford benzothiazole **5** (Scheme 6). Based on NMR spectrum of the reaction mixture, we supposed that product **5** was present, however its isolation failed.



Scheme 6.

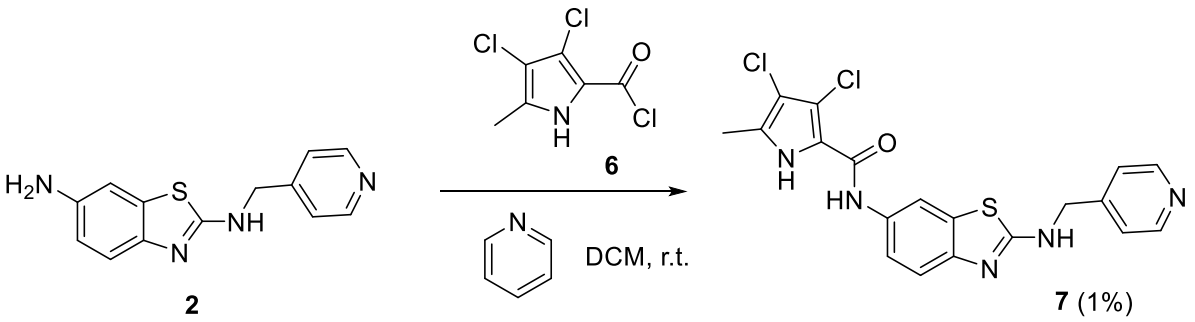
3.1.1.4 Synthesis of 3,4-dichloro-5methyl-*N*-(2-((pyridine-4-ylmethyl)amino) benzo[*d*]thiazol-6-yl-1*H*-pyrrole-2-carboxyamide

Chloride **6** was freshly prepared from 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid by reaction with 5.0 equivalents of 2.0 M oxalyl chloride in DCM (Scheme 7). Yield was 91%. The resultant product was used for the next step without further characterization.



Scheme 7.

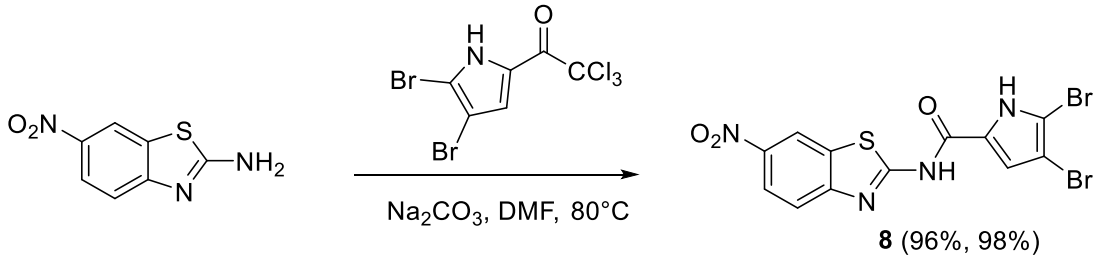
Furthermore, the amide **7** was obtained by the reaction of benzothiazole **2** with 1.1 equivalents of chloride **6** in presence of pyridine, using DCM as solvent (Scheme 8). The resultant amide **7** was purified, however, the final yield was only 1%.



Scheme 8.

3.1.2 Synthesis of 4,5-dibromo-*N*-(6-nitrobenzo[*d*]thiazol-2-yl)-1*H*-pyrrole-2-carboxamide

Due to the failure of the preparation of amide **7**, modified synthesis employing 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one (because of its better reactivity and stability than chloride **4**) was carried out. First step of the reaction sequence was condensation of 2-amino-6-nitrobenzothiazole with 1.1 equivalents of 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one in presence of 1.0 equivalent of sodium carbonate in DMF to afford amide **8** as essential compound for further reactions (Scheme 9). The reaction was repeated, yields were 96% and 98%. Structure was confirmed by ^{1}H NMR analysis: number of hydrogen is 6, amide hydrogen corresponds to singlet (δ 13.35), pyrrole hydrogen in position 1 to singlet (δ 13.03) and pyrrole hydrogen in carbon 3 to singlet (δ 7.58).

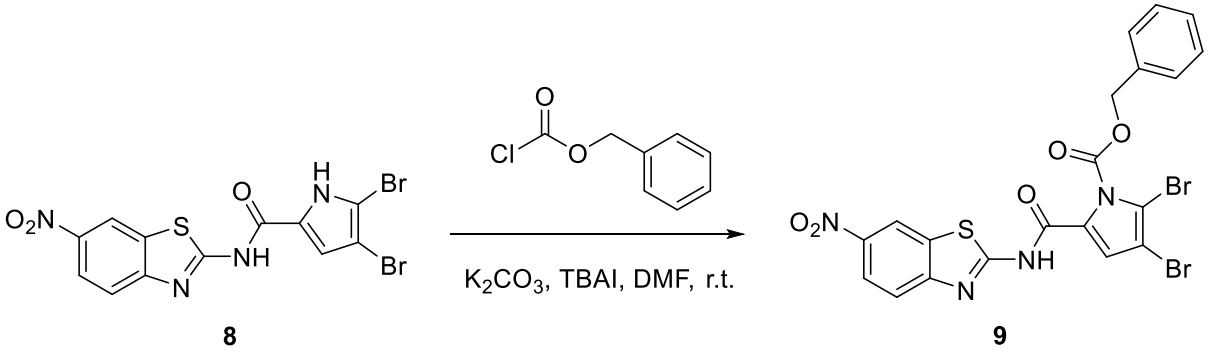


Scheme 9.

3.1.2.1 Synthesis of benzyl 2,3-dibromo-5-((6-nitrobenzo[*d*]thiazol-2-yl) carbamoyl)-1*H*-pyrrole-1-carboxylate

To protect the pyrrole nitrogen, 5.34 equivalents of benzyl chloroformate reacted with previously prepare amide **8** in presence of 5.34 equivalents of potassium carbonate and 1.07

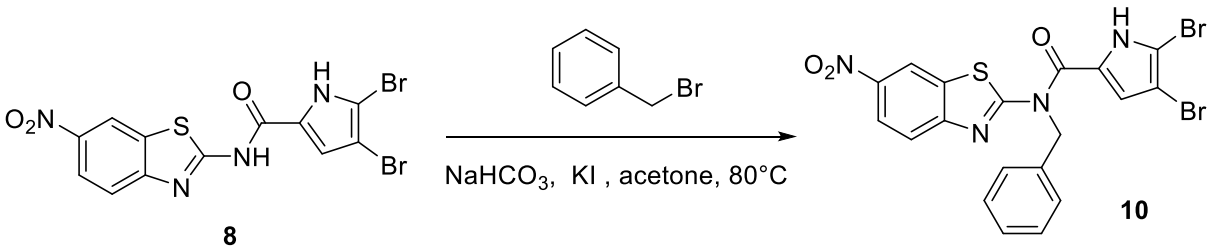
equivalents of tetrabutylammonium iodide in DMF (Scheme 10).[36] According to the synthetic plan, hydrogen of amide group should be replaced by different substituents, and so protection of pyrrole nitrogen was important. We supposed that benzyl chloroformate, because of its spatial arrangement, will be bound only and directly to nitrogen of pyrrole, not to amide nitrogen. However, NMR analysis shown that the final mixture was probably mixture of mono- and di- substituted product. Isolation of compound **9** failed.



Scheme 10.

3.1.2.2 Synthesis of ethyl 2-((2-(*N*-benzyl-4,5-dibromo-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-6-yl)amino)-2-oxoacetate

In view of the fact that protection of pyrrole nitrogen was not successful, compound **8** reacted directly with benzyl bromide in presence of sodium hydrogen carbonate and potassium iodide in acetone as solvent (Scheme 11). Reaction was repeated, employed equivalents and yields are summarized in Table 2.



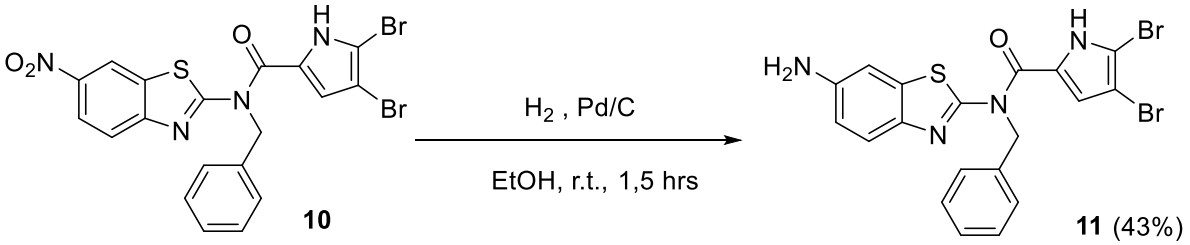
Scheme 11.

Table 2.

Formula	$C_{12}H_6Br_2N_4O_3S$	C_7H_7Br	$NaHCO_3$	KI	Yield (%)
MW	446,07	171,04	84,01	166,003	-
Eq 1st reaction	1	1	1	1	19
Eq 2nd reaction	1	1,5	2	1	60

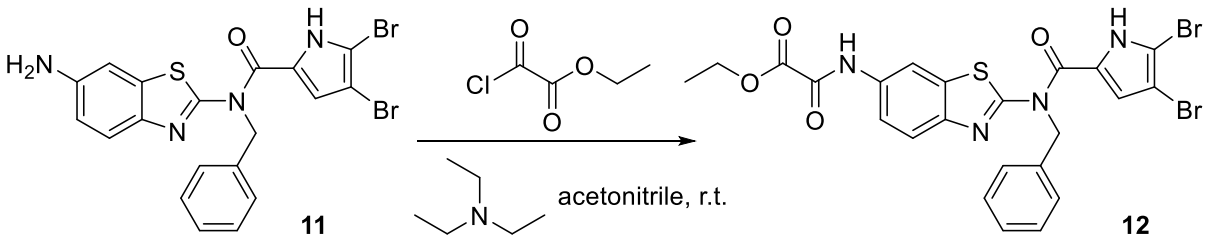
The structure of product **10** was confirmed by ^1H NMR analysis: pyrrole hydrogen in position 1 corresponds to doublet (δ 12.99), pyrrole hydrogen in carbon 3 to doublet (δ 7.11). In comparison to compound **8**, signal (δ 13.35) for amide hydrogen disappeared, however singlet representing two protons (δ 5.92) and multiplet representing five protons (δ 7.50-7.26) for benzyl group appeared.

The next step of the reaction sequence was the catalytic reduction of nitro group in position 6 of benzothiazole **10** with 0.05 equivalents of palladium on carbon as catalyst in hydrogen atmosphere and absolute ethanol as solvent (Scheme 12). Yield of product **11** was 43% and was used for the next step of reaction without further characterization.



Scheme 12.

Finally, aminobenzothiazole **11** underwent the reaction with 1.6 equivalents of 2.0 M ethyl oxalyl chloride in presence of 1.0 equivalent of triethylamine using acetonitrile as solvent (Scheme 13). Based on TLC and NMR analysis of the crude reaction mixture, product **12** appeared in final mixture in small quantity, however, isolation failed. Yield was 0%.

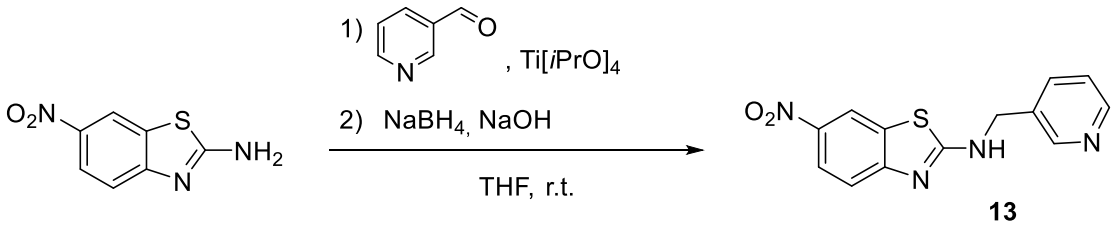


Scheme 13.

3.1.3 Synthesis of other benzothiazole derivatives

Other series of benzothiazoles were designed according to derivatives of 6-nitro-*N*-(pyridin-4-ylmethyl)benzo[*d*]thiazol-2-amine where 4-pyridinecarboxyaldehyde was replaced with 3-pyridinecarboxyaldehyde (Scheme 14) or *tert*-butyl (2-oxoethyl)carbamate (Scheme 15).

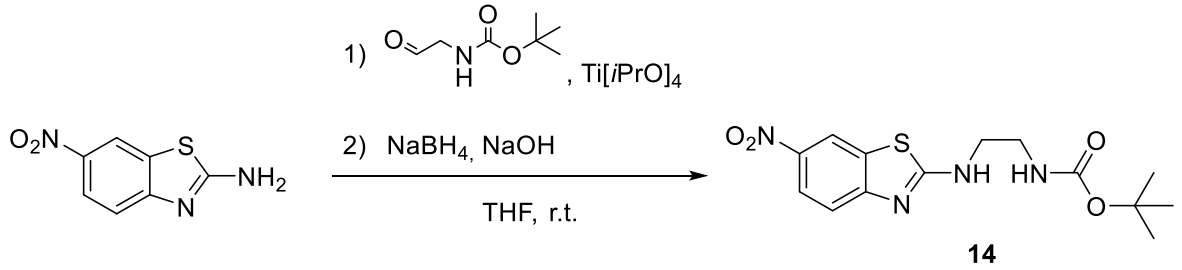
Synthesis of compound **13** was patterned on synthesis of compound **1**. In the first step, 2-amino-6-nitrobenzothiazole underwent the reaction with 1.2 equivalents of 3-pyridinecarboxyaldehyde in presence of 1.7 equivalents of titanium(IV) isopropoxide as catalyst in THF. Secondly, 3.2 equivalents of sodium borohydride were added to reduce the resultant imine. The reduction of imine was confirmed by TLC and reaction was quenched by 1.0 M sodium hydroxide solution (Scheme 14), however, product started to decompose immediately. Isolation of compound **13** failed.



Scheme 14.

The same general protocol was employed to synthetize benzothiazole **14**. 2-amino-6-nitrobenzothiazole reacted with 1.0 equivalent of *tert*-butyl (2-oxoethyl)carbamate in presence of 1.7 equivalents of titanium(IV) isopropoxide as catalyst in THF. Imine was reduced by addition of 1.6 equivalents of sodium borohydride and reaction was quenched by addition of

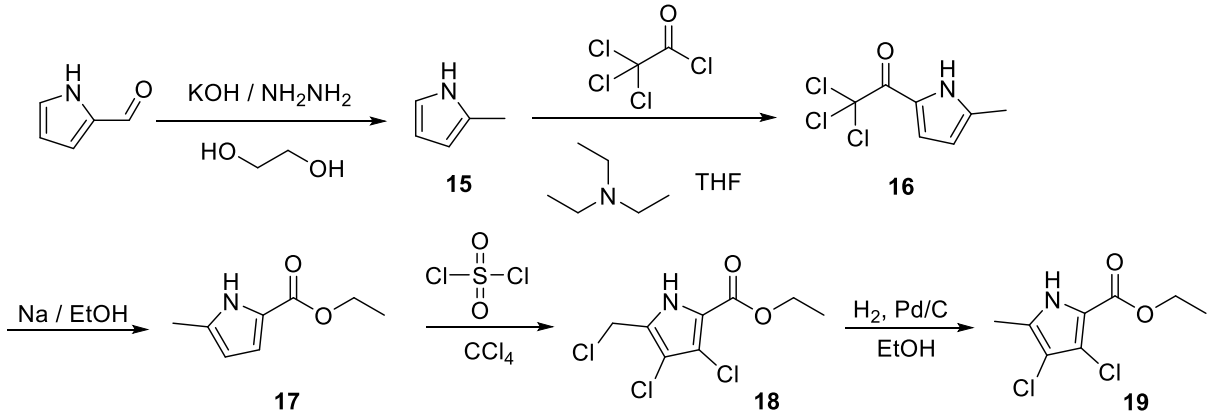
1.0 M sodium hydroxide solution (Scheme 15). Based on NMR analysis, we concluded that there was small amount of product **14** presented in the mixture, however purification of compound **14** failed.



Scheme 15.

3.2 Synthesis of 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate

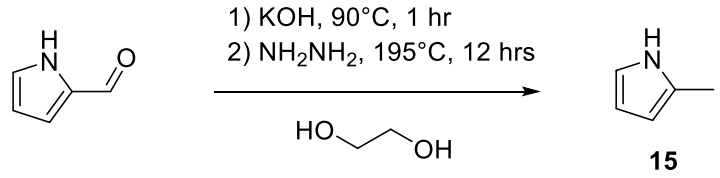
Synthesis of 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate (**19**) was designed and optimized for the preparation of tetrasubstituted pyrrole in large quantities (Scheme 16).



Scheme 16.

3.2.1 Synthesis of 2-methyl-1*H*-pyrrole

First step of reaction sequence was Wolff-Kishner reduction.[35] 1*H*-pyrrole-2-carbaldehyde reacted in presence of 1.7 equivalents of KOH in ethylene glycol, then 1.2 equivalents of hydrazine monohydrate were added to afford compound **15** as a yellow liquid (Scheme 17) that was used for the next step without further characterization.

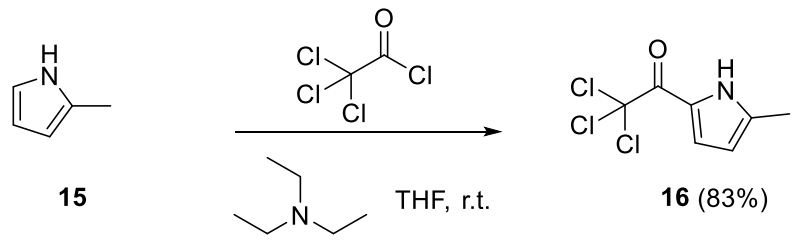


Scheme 17.

3.2.2 Synthesis of 2,2,2-trichloro-1-(5-methyl-1*H*-pyrrol-2-yl)ethan-1-one

Secondly, pyrrole **15** underwent nucleophilic aromatic substitution[35] in presence of 1.3 equivalents of 2,2,2-trichloroacetyl chloride, 1.2 equivalents of triethylamine and THF as a solvent (Scheme 18). Product **16** was isolated and yield was 83%.

^{1}H NMR analysis confirmed the structure: number of hydrogen is 6, hydrogen in position 1 corresponds to singlet (δ 9.81), methyl group in position 5 corresponds to singlet (δ 2.42).

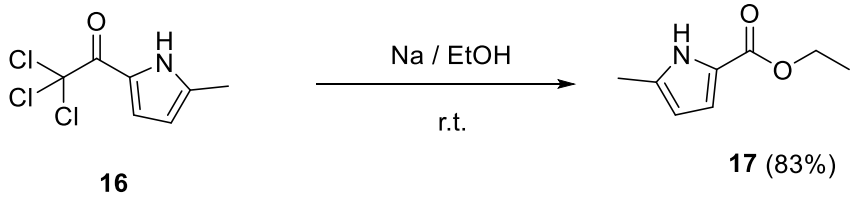


Scheme 18.

3.2.3 Synthesis of ethyl 5-methyl-1*H*-pyrrole-2-carboxylate

In the third step of reaction sequence, pyrrole **16** reacted with 1.2 equivalents of freshly sliced sodium in absolute ethanol to afford compound **17** (Scheme 19). Product was isolated with 83% yield.

The structure of product was confirmed by ^{1}H NMR analysis: number of hydrogen is 11, hydrogen in position 1 of pyrrole corresponds to singlet (δ 11.58), methyl group in position 5 corresponds to singlet (δ 2.21). In addition, -CH_2- group of ethyl ester appeared as quartet (δ 4.20) and CH_3- as triplet (δ 1.26).



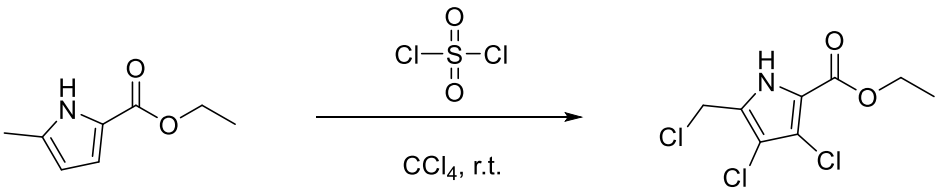
16 **17** (83%)

Scheme 19.

3.2.4 Synthesis of 3,4-dichloro-5-(chloromethyl)-1*H*-pyrrole-2-carboxylate

Furthermore, pyrrole **17** was chlorinated by 3.0 equivalents of freshly distilled sulfuryl chloride in tetrachloromethane as solvent (Scheme 20). Reaction was repeated twice, product **18** was isolated with yields 92% and 34%.

The structure was confirmed by ^{1}H NMR analysis: number of hydrogen is 8, hydrogen in position 1 of pyrrole corresponds to singlet (δ 9.86); ester CH_3- and -CH_2- groups correspond to triplet (δ 1.44) and quartet (δ 4.44), respectively. Signals for hydrogens in positions 3 and 4 disappeared. Singlet (δ 4.65) of methyl group is equal to two hydrogens instead of three, thus the chlorination is confirmed.



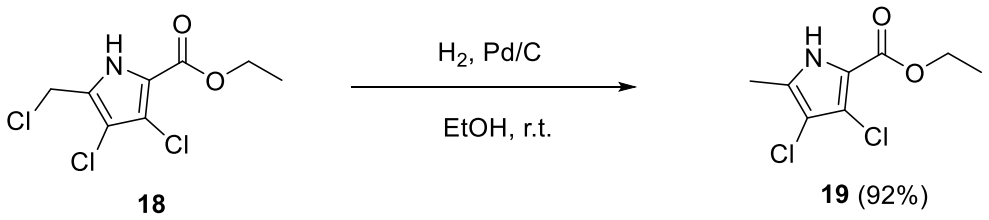
17 **18** (92%, 34%)

Scheme 20.

3.2.5 Synthesis of ethyl 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate

The last step of synthesis employed catalytic reduction of compound **18** with 0.05 equivalents of palladium on carbon as catalyst in absolute ethanol. The desired pyrrole **19** was isolated with 92% yield.

The structure of the final product was confirmed by NMR analysis: number of hydrogen is 9, hydrogen in position 1 of pyrrole corresponds to singlet (δ 9.81), ester CH_3- and -CH_2- groups correspond to triplet (δ 1.42) and quartet (δ 4.45), respectively. Dehalogenation of methyl group in position 5 of pyrrole was affirmed by singlet (δ 2.31) corresponding to 3 hydrogens instead of 2 hydrogens.

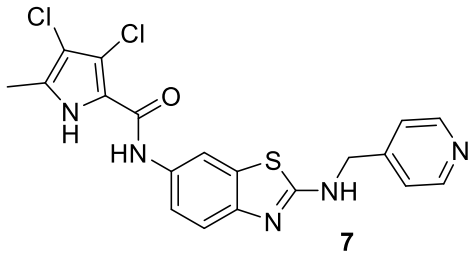


18 **19** (92%)

Scheme 21.

4 Conclusion

In my diploma thesis I focused on the synthesis of new derivatives of benzothiazoles. I prepared one final compound (**7**) which is not yet described in the literature and which was subjected to screening of its inhibitory activity. Other planned derivatives appeared in final mixtures in small amounts. Despite numerous attempts to purify final mixtures to gain the desired products, isolations failed.



7

In the second part of my diploma thesis, I prepared 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate in large quantity. Although synthesis was designed for milligram amounts, it run in large quantity as well with acceptable yields.

5 Experimental section

5.1 General procedures

All reagents were used as received from commercial source, Sigma Aldrich, without further purification unless otherwise indicated.

Melting points were determined on a Kofler block and are uncorrected.

Chemical shifts were measured as values δ in parts per million (ppm). ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on Bruker Ultrashield 400 PLUS spectrometer in DMSO-d_6 or $CDCl_3$ solution with TMS as an internal standard at 25°C. Data are represented in order: chemical shift (δ), multiplicity (s: singlet, d: doublet, t: triplet, dd: doublet of doublets, m: multiplet), coupling constant (Hz) and integrated intensity (in ^{1}H NMR).

Analytical TLC was performed on Merc silica gel (60 F_{254}) plates (0.25 mm) and components visualized with staining reagents or ultraviolet light.

Flash column chromatography was carried out on silica gel 60 (particle size 0.063-0.200 mm).

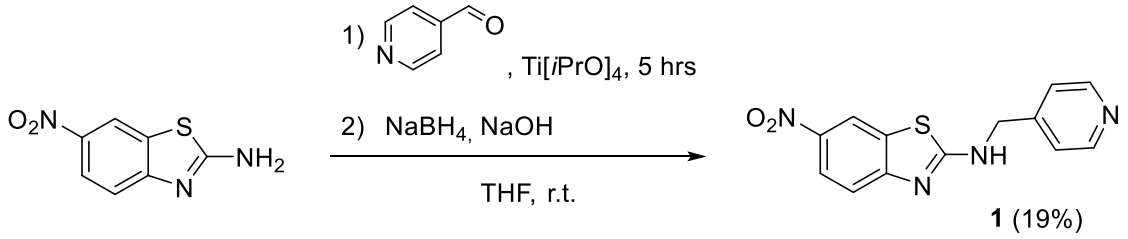
5.2 Synthetic procedures

5.2.1 Synthesis of 6-nitrobenzo[*d*]thiazole-2-amine derivatives

6-Nitro-*N*-(pyridin-4-ylmethyl)benzo[*d*]thiazol-2-amine

Formula: $C_{13}H_{10}N_4O_2S$

Molecular weight: 286,31 g.mol^{-1}



To a suspension of 2-amino-6-nitrobenzothiazole (3 g, 15.37 mmol) and titanium(IV) isopropoxide (7.74 mL, 26.13 mmol) in tetrahydrofuran (100 mL), 4-pyridinecarboxyaldehyde (1.74 mL, 18.44 mmol) was added. The mixture was stirred at room temperature for 5 hours followed by careful addition of sodium borohydride (0.930 g, 24.59 mmol). After 10 minutes, reaction was quenched by 0.4 M sodium hydroxide solution (60 mL). The mixture was filtered through Celite and the solvent was evaporated under reduced pressure. The crude product was recrystallized from ethanol. Compound **1** was isolated. Yield was 839 mg (19%).

Reaction was repeated according to preceding procedure. Yield was 1.2g (27%).

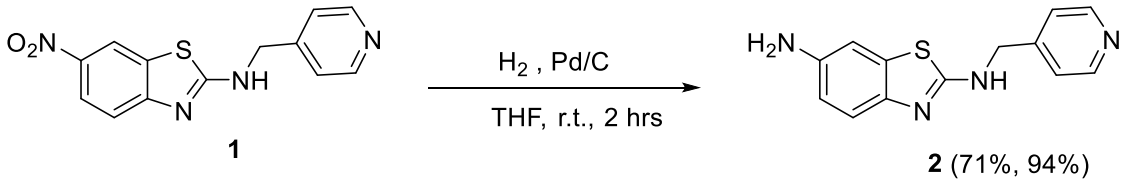
Crystals from first and second reaction were joined together (2.04 g) and recrystallization from dimethylformamide was made. Yield was 1.67g (82%).

Yellow crystals, mp over 200°C. **^{1}H NMR** (400 MHz, DMSO) δ 9.31 (s, 1H) 8.76 (d, *J* = 2.5 Hz, 1H), 8.58–8.48 (m, 2H), 8.11 (dd, *J* = 8.9 Hz, 2.5 Hz, 1H), 7.48 (d, *J* = 8.9 Hz, 1H), 7.43–7.33 (m, 2H), 4.73 (s, 2H).

N^2-(Pyridin-4-ylmethyl)benzo[*d*]thiazole-2,6-diamine

Formula: $C_{13}H_{12}N_4S$

Molecular weight: 256,33 g.mol^{-1}



To the solution of compound **1** (905 mg, 3 mmol) in THF (100 mL) was added 10% Pd/C (50 mg) and the reaction was stirred under hydrogen atmosphere at room temperature overnight. 1 mL of acetic acid was added and reaction was stirred for 2 hours. The catalyst was filtered out, the solvent was removed under reduced pressure to afford compound **2**. Yield was 579 mg (71%).

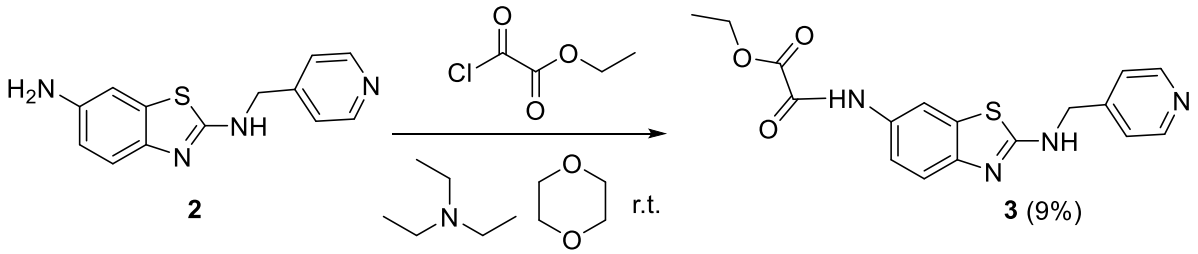
Reaction was repeated with compound **1** (286 mg, 1 mmol) according to preceding procedure. Yield was 240 mg (94%).

Grey-white crystals; mp 180°-183°C. Compound was used in next reaction step without further characterization.

Ethyl 2-oxo-2-((2-((pyridin-4-ylmethyl)amino)benzo[*d*]thiazol-6-yl)amino)acetate

Formula: $C_{17}H_{16}N_4O_3S$

Molecular weight: 356,40 g.mol^{-1}



To the mixture of compound **2** (329 mg, 1.3 mmol) and trimethylamine (0.12 mL, 1.3 mmol) in dioxane (20 mL) cooled on an ice bath, ethyl oxalyl chloride (0.14 mL, 1.3 mmol) was added dropwise. The mixture was stirred at room temperature overnight. 0.2 equivalent of ethyl oxalyl chloride (28.6 µL) was added. After 2 hours, reaction was stopped by addition of saturated aqueous sodium hydrogen carbonate (5 mL). Solvent was removed under reduced pressure, the residue dissolved in ethyl acetate (20 mL) was washed with saturated aqueous $NaHCO_3$ (2 x 20 mL), water (2 x 20 mL) and brine (1x 20 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude

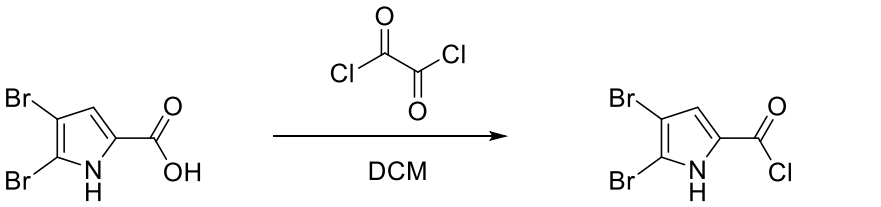
product was purified by flash column chromatography using dichloromethane/methanol (9:1) as eluent, for further purification by flash column chromatography toluene/2-propanol (4:1) was used as eluent. Compound **3** was isolated. Yield: 39 mg (9%)

Crystals; mp 191°-193°C. **^{1}H NMR** (400 MHz, DMSO) δ 10.79 (s, 1H), 8.64 (t, *J* = 6.0 Hz, 1H), 8.56–8.46 (m, 2H), 8.21–8.04 (m, 1H), 7.61–7.50 (m, 1H), 7.40–7.32 (m, 3H), 4.64 (d, *J* = 6.0 Hz, 2H), 4.31 (q, *J* = 7.1 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (MHz, DMSO) δ 166.0, 160.8, 155.1, 149.6, 149.4, 148.1, 131.3, 130.6, 122.1, 119.0, 118.0, 113.1, 62.3, 45.9, 13.8.

4,5-Dibromo-1*H*-pyrrole-2-carbonyl chloride

Formula: $C_5H_2Br_2ClNO$

Molecular weight: 287,34 g.mol^{-1}



4 (90%, 99%)

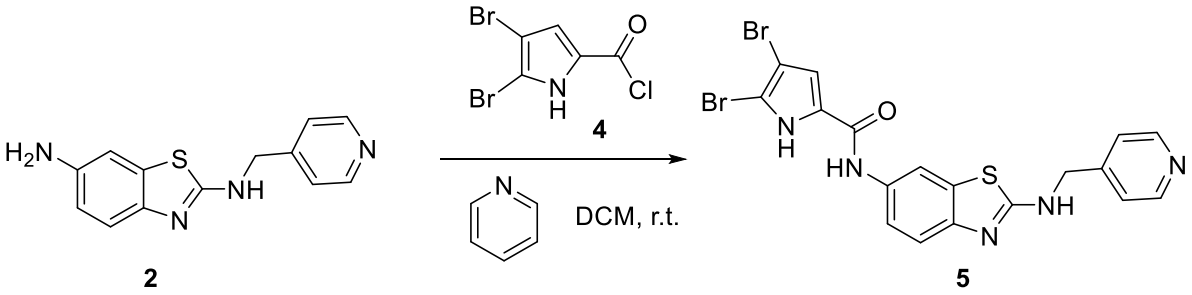
To a suspension of 4,5-dibromo-1*H*-pyrrole-2-carboxylic acid (230 mg, 0.86 mmol) in dichloromethane (20 mL), 2M oxalyl chloride (1.95 mL, 3.9 mmol) was added dropwise under argon atmosphere and mixture was stirred overnight. The solvent and oxalyl chloride were evaporated under reduced pressure to afford compound **4** as pink-brown crystals. Yield was 221 mg (90%). Compound was used in next reaction step without further purification and characterization.

Reaction was repeated with starting compound (1 g, 372 mmol) according to preceding procedure. Yield was 1.068 mg (99%).

4,5-Dibromo-*N*-(2-((pyridin-4-ylmethyl)amino)benzo[*d*]thiazol-6-yl)-1*H*-pyrrole-2-carboxamide

Formula: $C_{18}H_{13}Br_2N_5OS$

Molecular weight: 507,20 g.mol^{-1}

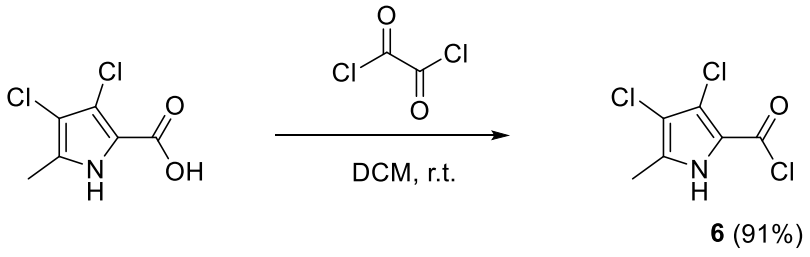


To a suspension of compound **2** (200mg, 0.78 mmol) in mixture of dichloromethane (10 mL) and pyridine (2 mL), compound **4** was added under argon atmosphere and mixture was stirred overnight. Solvents were removed under reduced pressure, the residue dissolved in ethyl acetate (20 mL) was washed with saturated aqueous $NaHCO_3$ (3 x 20 mL) and brine (1x 20 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography using toluene/2-propanol (3/1) as eluent to afford **5** as yellow-orange crystals. Yield was 2 mg (0.5%). No further characterization.

3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carbonyl chloride

Formula: $C_6H_4Cl_3NO$

Molecular weight: 212,45 g.mol^{-1}



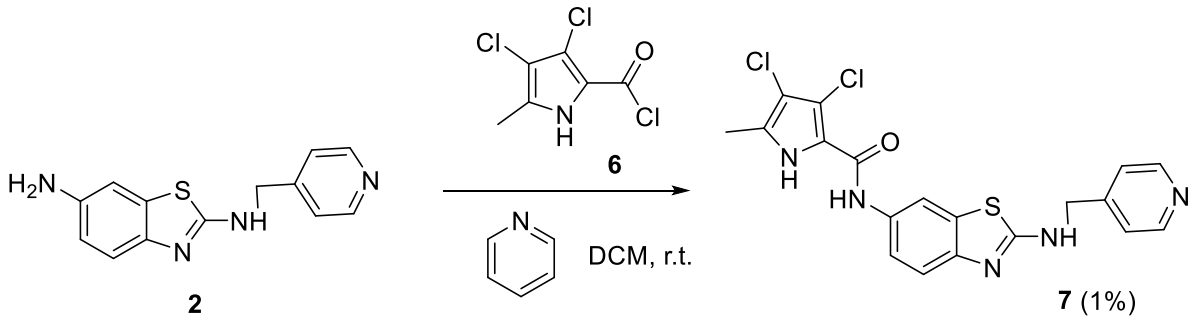
6 (91%)

To a suspension of 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid (225 mg, 1.16 mmol) in dichloromethane (20 mL), 2M oxalyl chloride (2.9 mL, 3.9 mmol) was added dropwise under argon atmosphere and mixture was stirred overnight. The solvent and oxalyl chloride were evaporated under reduced pressure to afford compound **6** as brown crystals. Yield was 0.223 mg (91%). Compound was used in next reaction step without further purification and characterization.

3,4-Dichloro-5methyl-*N*-(2-((pyridine-4-ylmethyl)amino)benzo[*d*]thiazol-6-yl-1*H*-pyrrole-2-carboxyamide

Formula: $C_{19}H_{15}Cl_2N_5OS$

Molecular weight: 432,32 $g.mol^{-1}$



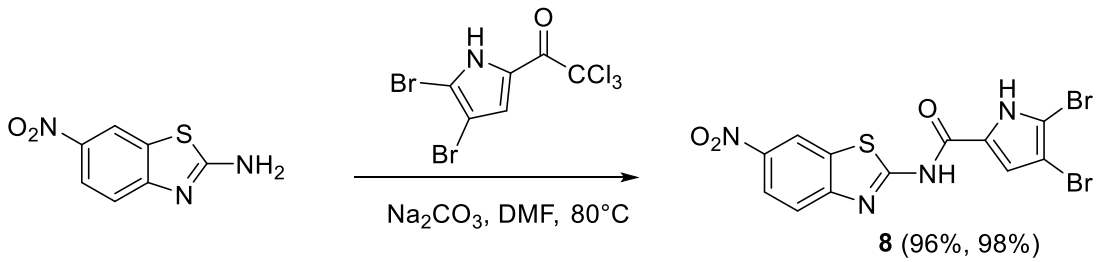
To a suspension of compound **2** (226 mg, 1 mmol) in mixture of dichloromethane (15 mL) and pyridine (2 mL), compound **6** was added under argon atmosphere and mixture was stirred overnight. Solvents were removed under reduced pressure, the residue dissolved in ethyl acetate (20 mL) was washed with saturated aqueous $NaHCO_3$ (2 x 20 mL) and brine (2x 20 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography using dichloromethane/methanol (9/1) as eluent to afford **7** as grey crystals. Yield was 2.2 mg (1%).

Grey crystals; mp over 200°C. **^{1}H NMR** (400 MHz, DMSO) δ 12.15 (s, 1H), 9.39 (s, 1H), 8.71–8.39 (m, 2H), 8.08 (d, *J* = 2.1 Hz, 1H), 7.57–7.24 (m, 4H), 4.46 (d, *J* = 5.9 Hz, 2H), 2.23 (s, 3H). **^{13}C NMR** (MHz, DMSO) δ 165.6, 157.0, 149.6, 148.7, 148.2, 132.4, 130.7, 127.8, 122.1, 119.7, 118.7, 118.0, 112.7, 110.9, 108.3, 45.9, 10.8.

4,5-Dibromo-*N*-(6-nitrobenzo[*d*]thiazol-2-yl)-1*H*-pyrrole-2-carboxamide

Formula: $C_{12}H_6Br_2N_4O_3S$

Molecular weight: 446,07 $g.mol^{-1}$



8 (96%, 98%)

To a solution of 2-amino-6-nitrobenzothiazole (0.527 g, 2,70 mmol) and sodium carbonate (0,286 g, 2,70 mmol) in DMF (20 mL), 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one (1,1 g, 2,98 mmol) was added and mixture was stirred at 80°C overnight. Solvent was evaporated to afford compound **8** as brown-grey compound. Yield was 1,15 g (96%).

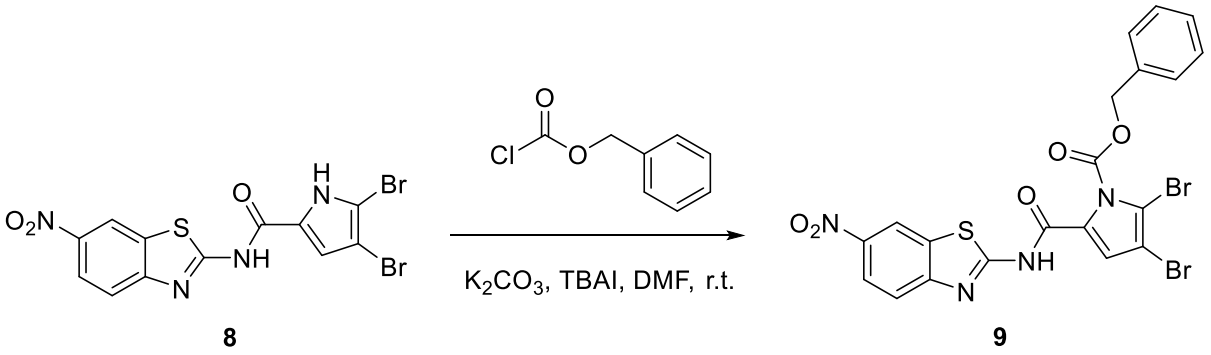
Reaction was repeated with starting compound (227 mg, 1,16 mmol) according to preceding procedure. Yield was 510 mg (98%).

Brown-grey compound. **^{1}H NMR** (400 MHz, DMSO) δ 13.35 (s, 1H), 13.03 (s, 1H), 9.09 (d, *J* = 2.5 Hz, 1H), 8.31 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.92 (d, *J* = 8.9 Hz, 1H), 7.58 (s, 1H).

Benzyl 2,3-dibromo-5-((6-nitrobenzo[*d*]thiazol-2-yl)carbamoyl)-1*H*-pyrrole-1-carboxylate

Formula: $C_{20}H_{12}Br_2N_4O_5S$

Molecular weight: 580,21 g.mol^{-1}



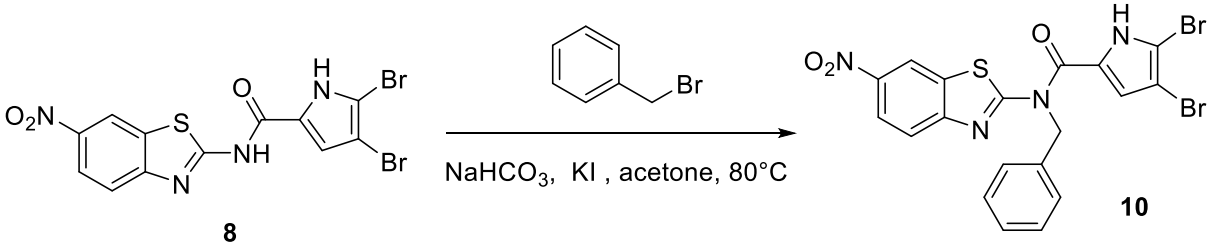
8 **9**

To a suspension of a compound **8** (477 mg, 1.07 mmol) in DMF (15 mL), benzyl chloroformate (0.82 mL, 5.71 mmol), potassium carbonate (789 mg, 5.71 mmol) and TBAI (395 mg, 1.07 mmol) were added sequentially and mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, the residue dissolved in ethyl acetate (20 mL), washed with saturated aqueous $NaHCO_3$ (2 x 20 mL) and brine (1 x 20 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford **9** (483 mg) as yellow crystals. Yield was 483 mg (78%); melting point over 200°C. No further characterization.

***N*-Benzyl-4,5-dibromo-*N*-(6-nitrobenzo[*d*]thiazol-2-yl)-1*H*-pyrrole-2-carboxamide**

Formula: $C_{19}H_{12}Br_2N_4O_3S$

Molecular weight: 580,21 g.mol^{-1}



8 **10**

To a suspension of **8** (223 mg, 0.5 mmol), sodium hydrogen carbonate (42 mg, 0.5 mmol) and potassium iodide (83 mg, 0.5 mmol) in dry acetone (10 mL), benzyl bromide (0.06 mL, 0.5 mmol) was added under argon atmosphere and mixture was stirred at 80°C for 2 days. The solvent was evaporated under reduced pressure and crude product was purified by flash column chromatography using pure dichloromethane as eluent. Yield was 50 mg (19%).

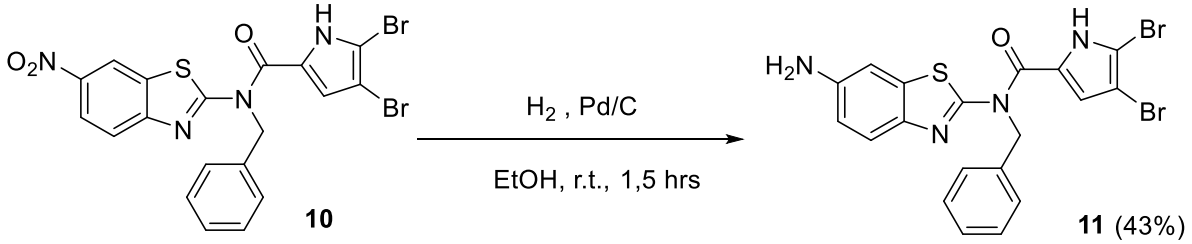
Reaction was repeated with compound **8** (571 mg, 1.28 mmol) according to preceding procedure. Yield was 406 mg (60 %).

Amorphous compound. **^{1}H NMR** (400 MHz, DMSO) δ 12.99 (d, *J* = 2.7 Hz, 1H), 8.95 (d, *J* = 2.4 Hz, 1H), 8.31 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.75 (d, *J* = 9.0 Hz, 1H), 7.50–7.26 (m, 5H), 7.11 (d, *J* = 2.7 Hz, 1H), 5.92 (s, 2H).

N-(6-Aminobenzo[*d*]thiazol-2-yl)-*N*-benzyl-4,5-dibromo-1*H*-pyrrole-2-carboxamide

Formula: $C_{19}H_{14}Br_2N_4OS$

Molecular weight: 506,22 g.mol^{-1}

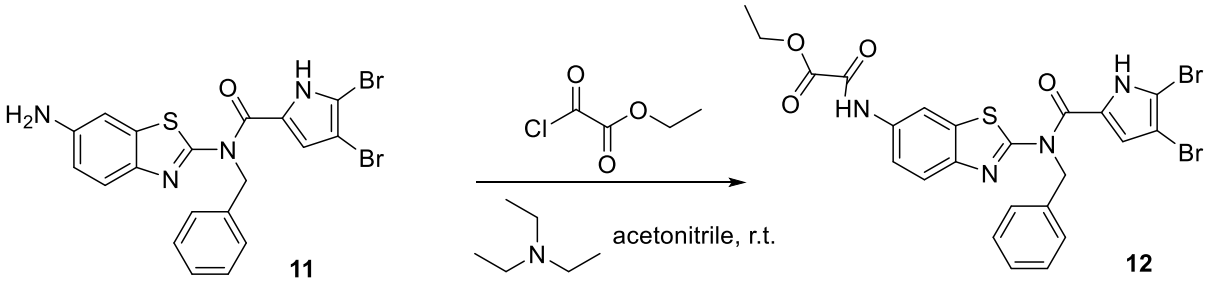


To the solution of compound **10** (215 mg, 0,04 mmol) in absolute ethanol (150 mL), 10% Pd/C (11 mg) was added and the reaction was stirred under hydrogen atmosphere at room temperature for 1.5 hours. The catalyst was filtered out and THF was evaporated under reduced pressure to afford **11** (88 mg) as grey compound. Yield was 88 mg (43%). Compound was used in next reaction step without further purification and characterization.

Ethyl 2-((2-(*N*-benzyl-4,5-dibromo-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-6-yl)amino)-2-oxoacetate

Formula: $C_{23}H_{18}Br_2N_4O_4S$

Molecular weight: 606,29 g.mol^{-1}

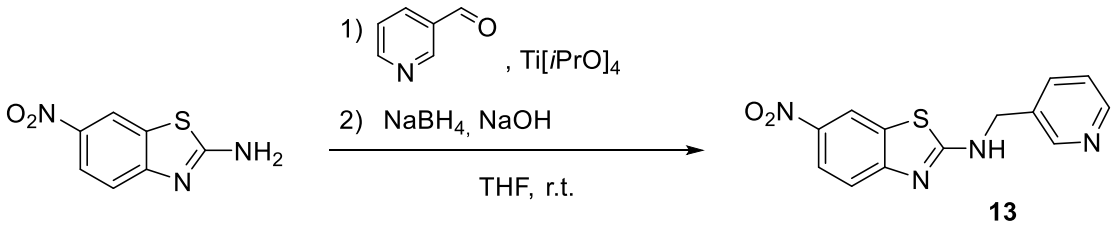


To a solution of compound 11 (88 mg, 0.17 mmol) and triethylamine (0.03 mL, 0.17 mmol) in acetonitrile (20 mL) cooled on an ice bath, ethyl oxalyl chloride was added dropwise. The mixture was stirred at room temperature overnight. 0,5 equivalent of ethyl oxalyl chloride (0.01 mL, 0.08 mmol) was added and reaction run overnight. The solvent was evaporated under reduced pressure and column chromatography and the crude product was purified by flash column chromatography using dichloromethane/methanol (50/1) as eluent. Isolation failed.

5.2.1.12 6-Nitro-*N*-(pyridin-3-ylmethyl)benzo[*d*]thiazol-2-amine

Formula: $C_{13}H_{10}N_4O_2S$

Molecular weight: 286,31 g.mol^{-1}



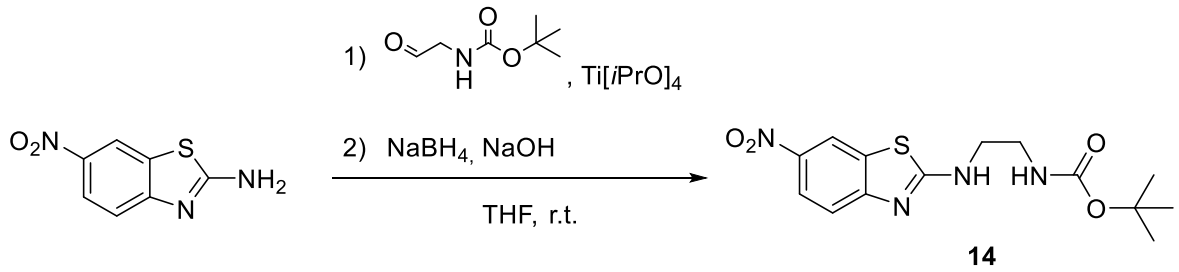
According to general procedure A, to a suspension of 2-amino-6-nitrobenzothiazole (6 g, 30.74 mmol) and titanium(IV) isopropoxide (15.47 mL, 52.26 mmol) in dry tetrahydrofuran (120 mL), nicotinealdehyde (3,48 mL, 36,89 mmol) was added. The mixture

was stirred at room temperature overnight followed by careful addition of sodium borohydride (1.86 g, 49.18 mmol). Reaction run overnight. 1.6 equivalents of $NaBH_4$ were added (1.86 g). After 2 hours, reaction was quenched by 1.0 M sodium hydroxide solution (50 mL). THF was evaporated under reduced pressure, recrystallization from ethanol was made. Crystals of starting compound were filtered out. Product decomposed.

Tert-Butyl (2-((6-nitrobenzo[*d*]thiazol-2-yl)amino)ethyl)carbamate

Formula: $C_{14}H_{18}N_4O_4S$

Molecular weight: 338,38 g.mol^{-1}



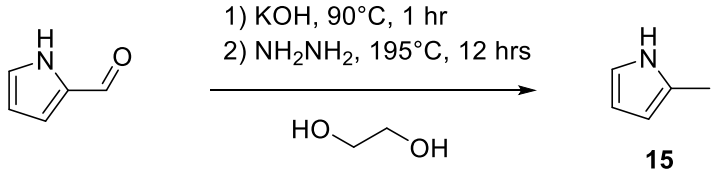
According to general procedure A, to a suspension of 2-amino-6-nitrobenzothiazole (195 mg, 1 mmol) and titanium(IV) isopropoxide (0.50 mL, 1.7 mmol) in tetrahydrofuran (15 mL), *terc*-butyl (2-oxoethyl)carbamate (159 mg, 1 mmol) was added. The mixture was stirred at room temperature 2 days followed by careful addition of sodium borohydride (60.5 mg, 1.6 mmol). Reaction run overnight and was quenched by addition of 1M sodium hydroxide (40 mL). Mixture was filtered through Celite and THF was evaporated under reduced pressure. Recrystallization from ethanol was made. Crystals were dissolved in mixture of dichloromethane/methanol (9/1) and undissolved inorganic impurities were filtered out. Unsuccessful attempt to purify the crude product was by flash column chromatography using dichloromethane/methanol (9/1) as eluent. Isolation failed.

5.2.2 Synthesis of ethyl 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate

2-Methyl-1*H*-pyrrole

Formula: C_5H_7N

Molecular weight: 81,12 g.mol^{-1}

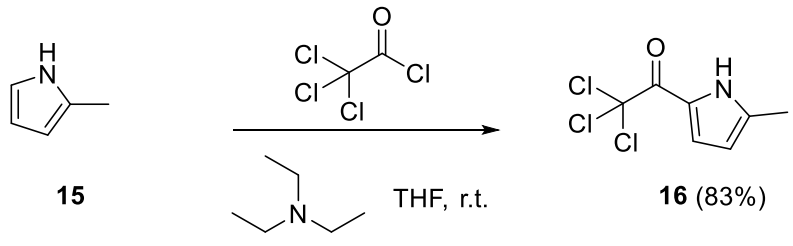


To a solution of 1*H*-pyrrole-2-carbaldehyde (51.226 g, 539 mmol) in ethylene glycol (300 mL), hydrazine was added dropwise and mixture was stirred at 90°C for 1 hour. Potassium hydroxide (51.380 g, 916 mmol) was added and mixture was stirred at 195°C overnight. The crude product was distillated, dissolved in dichloromethane and washed with saturated aqueous $NaHCO_3$ (2 x 100 mL) and with brine (1 x 100 mL). Dichloromethane was evaporated carefully under reduced pressure to afford **15** as yellow liquid. Compound was used in next step of reaction without further characterization.

2,2,2-Trichloro-1-(5-methyl-1*H*-pyrrol-2-yl)ethan-1-one

Formula: $C_7H_6Cl_3NO$

Molecular weight: 226,48 g.mol^{-1}



To a solution of compound **15** (43.695 g, 539 mmol) and triethylamine (89.60 mL, 646 mmol) in THF (200 mL) cooled on an ice bath at 0°C, 2,2,2-trichloroacetyl chloride (78.16 mL, 700 mmol) was added dropwise. Ice bath was removed after 1.5 hour and mixture was stirred at room temperature overnight. Saturated aqueous $NaHCO_3$ (60 mL) was added to

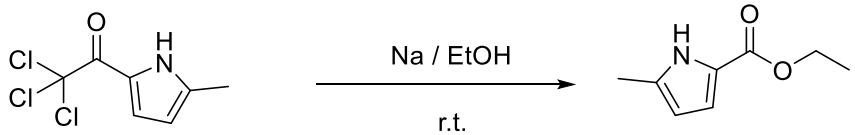
mixture till white precipitate started to appear, the crude product was filtered out and used in next step without further purification. Yield was 100.91 g (83%).

White crystals; mp 87-90 °C, lit.[37] 104-106 °C, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 7.38–7.32 (m, 1H), 6.13 (s, 1H), 2.42 (s, 3H).

Ethyl 5-methyl-1*H*-pyrrole-2-carboxylate

Formula: $C_8H_{11}NO_2$

Molecular weight: 153,18 g.mol^{-1}



16 **17** (83%)

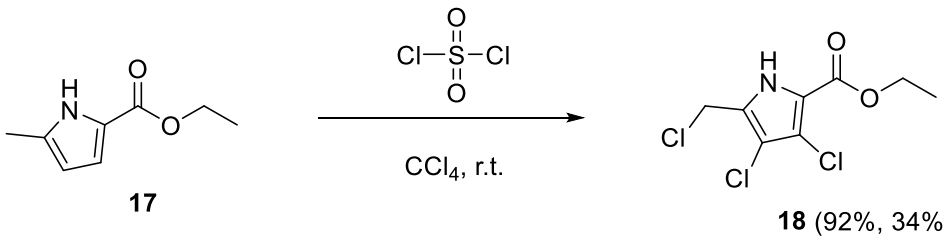
Sodium (12.29 g, 535 mmol) was dissolved in absolute ethanol (400 mL) cooled on an ice bath at 0°C under argon atmosphere. 16 (100.91 g, 446 mmol) was added and mixture was stirred at room temperature overnight. Ethanol was evaporated under reduced pressure, and the residue was dissolved in mixture of ethyl acetate (100 mL) and water (100 mL). Organic phase was washed with brine (100 mL), dried over Na_2SO_4, filtered and ethyl acetate was evaporated under reduced pressure to afford **17** as brown-white solid. Yield was 55.355 g (83 %).

Brown-white amorphous compound; lit[38] 94-96 °C, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.58 (s, 1H), 6.73–6.55 (m, 1H), 5.95–5.73 (m, 1H), 4.20 (q, *J* = 7.1, 2H), 2.21 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H).

Ethyl 3,4-dichloro-5-(chloromethyl)-1*H*-pyrrole-2-carboxylate

Formula: $C_8H_8Cl_3NO_2$

Molecular weight: 256,51 g.mol^{-1}



17 **18** (92%, 34%)

Compound **17** (20.36 g, 133 mmol) was dissolved in tetrachloromethane (300 mL) cooled on ice bath at 0°C under argon atmosphere. Freshly distillated sulfuryl chloride (32.32 mL, 399 mmol) was added dropwise, ice bath was removed after 1 hour and mixture was stirred at room temperature as long as precipitate appeared. The precipitate was filtered out and recrystallized from dichloromethane. Brown-white crystals; yield was 30.44 g (92%).

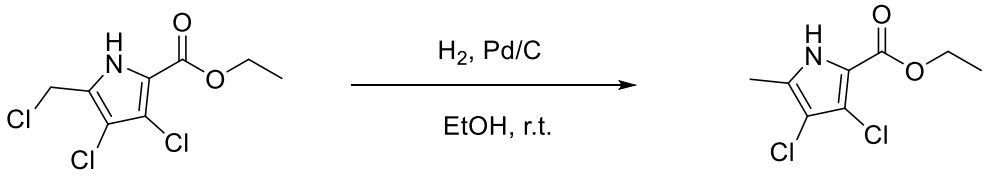
Reaction was repeated with compound **17** (35 g, 229 mmol) according to preceding procedure. Yield was 20 g (34%).

Brown-white crystals; mp 145-147 °C, lit[39] 158-159 °C, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.86 (s, 1H), 4.65 (s, 2H), 4.44 (q, *J* = 7.1 Hz, 2H), 1.44 (t, *J* = 7.1 Hz, 3H).

Ethyl 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate

Formula: $C_8H_9Cl_2NO_2$

Molecular weight: 222,07 g.mol^{-1}



18 **19** (92%)

To a solution of compound **18** (14,36 g, 56 mmol) in absolute ethanol (200 mL), 10% Pd/C (718 mg) was added and the reaction was stirred under hydrogen atmosphere for 2 days. The catalyst was filtered out and crude product was recrystallized from ethanol. Brown-white solid; yield was 11.386 g (92%).

Brown-white amorphous compound; lit.[39] 161-162 °C, **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 4.45 (q, *J* = 7.2 Hz, 2H), 2.31 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H).

6 List of abbreviations

ΔLk	Linking difference
DCM	Dichloromethane
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E. Coli	*Escherichia coli*
GyrA	Gyrase A subunit
GyrB	Gyrase B subunit
Lk	Linking number
Lk_0	Linking number of completely relaxed DNA
NAPs	Nucleoid-associated proteins
NMR	Nuclear magnetic resonance
ParC	Topoisomerase IV C subunit
ParE	Topoisomerase IV E subunit
TBAI	Tetrabutylammonium iodide
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
Tw	Twist number
Wr	Writhe number

7 References

[1] GOULD, Kate. Antibiotics: from prehistory to the present day. *Journal of Antimicrobial Chemotherapy* [online]. 2016, **71**(3), 572-575 [cit. 2016-06-26]. DOI: 10.1093/jac/dkv484. ISSN 0305-7453. Available from: http://www.jac.oxfordjournals.org/lookup/doi/10.1093/jac/dkv484

[2] Wollman AJM, Nudd R, Hedlund EG, Leake MC. 2015 From *Animaculum* to single molecules: 300 years of the light microscope. *Open Biol*. **5**: 150019. http://dx.doi.org/10.1098/rsob.150019

[3] BLEVINS, Steve M. a Michael S. BRONZE. Robert Koch and the 'golden age' of bacteriology. *International Journal of Infectious Diseases*[online]. 2010, **14**(9), e744-e751 [cit. 2016-06-26]. DOI: 10.1016/j.ijid.2009.12.003. ISSN 12019712. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1201971210023143

[4] VALENT, Peter, Bernd GRONER, Udo SCHUMACHER, et al. Paul Ehrlich (1854-1915) and His Contributions to the Foundation and Birth of Translational Medicine. *Journal of Innate Immunity* [online]. 2016-3-21, **8**(2), 111-120 [cit. 2016-06-26]. DOI: 10.1159/000443526. ISSN 1662-811x. Available from: http://www.karger.com/?doi=10.1159/000443526

[5] LLOYD, Nicholas C., Hugh W. MORGAN, Brian K. NICHOLSON a Ron S. RONIMUS. The Composition of Ehrlich's Salvarsan: Resolution of a Century-Old Debate. *Angewandte Chemie International Edition*[online]. 2005, **44**(6), 941-944 [cit. 2016-07-26]. DOI: 10.1002/anie.200461471. ISSN 1433-7851. Available from: http://doi.wiley.com/10.1002/anie.200461471

[6] AMINOV, Rustam I. A Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future. *Frontiers in Microbiology*[online]. 2010, **1**, - [cit. 2016-06-26]. DOI: 10.3389/fmicb.2010.00134. ISSN 1664-302x. Available from: http://journal.frontiersin.org/article/10.3389/fmicb.2010.00134/abstract

[7] NEČAS, Oldřich. *Obecná biologie pro lékařské fakulty*. 3. přeprac. vyd., V nakl. H & H 1. vyd. Jinočany: H & H, 2000. ISBN 80-86022-46-3.

[8] ALBERTS, Bruce. *Základy buněčné biologie: úvod do molekulární biologie buňky*. 2. vyd. Přeložil Arnošt KOTYK. Ústí nad Labem: Espero Publishing, 2004. ISBN 80-902906-2-0.

[9] KOČÁREK, Eduard. *Genetika: obecná genetika a cytogenetika, molekulární biologie, biotechnologie, genomika*. 2. vyd. Praha: Scientia, 2008. Biologie pro gymnázia. ISBN 978-80-86960-36-4.

[10] DNA. *Wikipedia: the free encyclopedia.* [online]. 2001- [cit. 2015-01-23]. Accessible from: http://en.wikipedia.org/wiki/DNA.

[11] THANBICHLER, Martin, Sherry C. WANG a Lucy SHAPIRO. The bacterial nucleoid: A highly organized and dynamic structure. *Journal of Cellular Biochemistry* [online]. 2005, **96**(3), 506-521 [cit. 2016-07-03]. DOI: 10.1002/jcb.20519. ISSN 0730-2312. Available from: http://doi.wiley.com/10.1002/jcb.20519

[12] DILLON, SC; DORNAN CJ. Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. *Nature Reviews Microbiology*. 8, 3 **185-195**, Mar. 2010. ISSN: 17401526

[13] BROWN D, McCALLUS D. Plasmid relationships and applications. *Salem Press Encyclopedia Of Health* [serial online]. January 2015; Available from: Research Starters, Ipswich, MA. Accessed July 3, 2016

[14] BENNER D, NESS B. Chromosome structure. *Salem Press Encyclopedia of Health* [serial online]. January 2015; Available from: Research Starters, Ipswich, mA. Accessed July 3, 2016

[15] WANNER, G. a H. FORMANEK. A New Chromosome Model. *Journal of Structural Biology* [online]. 2000, **132**(2), 147-161 [cit. 2016-07-03]. DOI: 10.1006/jsbi.2000.4310. ISSN 10478477. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1047847700943107

[16] Structure of the Double Helix. *GeneEd.* [online]. 2012- [cit. 2015-04-12]. Accessible from: http://geneed.nlm.nih.gov/topic_subtopic.php?tid=15&sid=16.

[17] MIRKIN, Sergei M. DNA Topology: Fundamentals. *eLS* [online]. May 2001, John Wiley & Sons Ltd, Chichester. DOI: 10.1038/npg.els.0001038. Accessible from: http://www.els.net

[18] DEWEESE, Joseph E., Michael A. OSHEROFF a Neil OSHEROFF. DNA topology and topoisomerases. *Biochemistry and Molecular Biology Education* [online]. 2009, **37**(1), 2-10 [cit. 2016-07-03]. DOI: 10.1002/bmb.20244. ISSN 14708175. Available from: http://doi.wiley.com/10.1002/bmb.20244

[19] NEUMAN, K. C. Single-molecule Measurements of DNA Topology and Topoisomerases. *Journal of Biological Chemistry* [online]. 2010,**285**(25), 18967-18971

[cit. 2016-07-03]. DOI: 10.1074/jbc.R109.092437. ISSN 0021-9258. Available from: http://www.jbc.org/cgi/doi/10.1074/jbc.R109.092437

[20] GILBERT, Nick a James ALLAN. Supercoiling in DNA and chromatin. *Current Opinion in Genetics & Development* [online]. 2014, **25**, 15-21 [cit. 2016-07-03]. DOI: 10.1016/j.gde.2013.10.013. ISSN 0959437x. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0959437X13001500

[21] BATES, Andrew D a Anthony MAXWELL. DNA topology: Topoisomerases keep it simple. *Current Biology* [online]. 1997, **7**(12), R778-R781 [cit. 2016-07-26]. DOI: 10.1016/S0960-9822(06)00403-9. ISSN 09609822. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0960982206004039

[22] TIMSIT, Y. Local sensing of global DNA topology: from crossover geometry to type II topoisomerase processivity. *Nucleic Acids Research* [online]. 2011, **39**(20), 8665-8676 [cit. 2016-07-26]. DOI: 10.1093/nar/gkr556. ISSN 0305-1048. Available from: http://nar.oxfordjournals.org/lookup/doi/10.1093/nar/gkr556

[23] CHAMPOUX J. DNA TOPOISOMERASES: Structure, Function, and Mechanism. *Annual Review Of Biochemistry* [serial online]. July 2001;70(1):369. Available from: Academic Search Complete, Ipswich, MA. Accessed July 6, 2016.

[24] BARANELLO, Laura, Fedor KOUZINE a David LEVENS. DNA Topoisomerases. *Transcription* [online]. 2014, **4**(5), 232-237 [cit. 2016-07-06]. DOI: 10.4161/trns.26598. ISSN 2154-1264. Available from: http://www.tandfonline.com/doi/abs/10.4161/trns.26598

[25] FORTERRE, Patrick, Simonetta GRIBALDO, Danièle GADELLE a Marie-Claude SERRE. Origin and evolution of DNA topoisomerases. *Biochimie* [online]. 2007, **89**(4), 427-446 [cit. 2016-07-06]. DOI: 10.1016/j.biochi.2006.12.009. ISSN 03009084. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0300908407000041

[26] COLLIN, Frédéric, Shantanu KARKARE a Anthony MAXWELL. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Applied Microbiology and Biotechnology* [online]. 2011, **92**(3), 479-497 [cit. 2016-07-06]. DOI: 10.1007/s00253-011-3557-z. ISSN 0175-7598. Available from: http://link.springer.com/10.1007/s00253-011-3557-z

[27] HARTL, Jiří. *Farmaceutická chemie IV*. Praha: Karolinum, 2006. ISBN 80-246-1169-4.

[28] CIULLA-BOHLING R. Quinolone antibiotics. *Salem Press Encyclopedia Of Health* [serial online]. January 2015; Available from: Research Starters, Ipswich, MA. Accessed July 16, 2016.

[29] ZAFFIRI, Lorenzo, Jared GARDNER a Luis H. TOLEDO-PEREYRA. History of Antibiotics: From Fluoroquinolones to Daptomycin (Part 2). *Journal of Investigative Surgery* [online]. 2013, **26**(4), 167-179 [cit. 2016-07-16]. DOI: 10.3109/08941939.2013.808461. ISSN 0894-1939. Available from: http://www.tandfonline.com/doi/full/10.3109/08941939.2013.808461

[30] HEIDE, Lutz. New aminocoumarin antibiotics as gyrase inhibitors. *International Journal of Medical Microbiology* [online]. 2014, **304**(1), 31-36 [cit. 2016-07-16]. DOI: 10.1016/j.ijmm.2013.08.013. ISSN 14384221. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1438422113001379

[31] DYSON, Lauren, Anthony D. WRIGHT, Kelly A. YOUNG, Jennette A. SAKOFF a Adam MCCLUSKEY. Synthesis and anticancer activity of focused compound libraries from the natural product lead, oroidin.*Bioorganic & Medicinal Chemistry* [online]. 2014, **22**(5), 1690-1699 [cit. 2016-07-17]. DOI: 10.1016/j.bmc.2014.01.021. ISSN 09680896. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0968089614000492

[32] RASAPALLI, Sivappa, Venkatreddy KUMBAM, Abasaheb N. DHAWANE, James A. GOLEN, Carl J. LOVELY a Arnold L. RHEINGOLD. Total syntheses of oroidin, hymenidin and clathrodin. *Organic & Biomolecular Chemistry* [online]. 2013, **11**(25), 4133- [cit. 2016-07-17]. DOI: 10.1039/c3ob40668g. ISSN 1477-0520. Available from: http://xlink.rsc.org/?DOI=c3ob40668g

[33] TOMAŠIČ, Tihomir, Sotirios KATSAMAKAS, Žiga HODNIK, et al. Discovery of 4,5,6,7-Tetrahydrobenzo[1,2- d]thiazoles as Novel DNA Gyrase Inhibitors Targeting the ATP-Binding Site. *Journal of Medicinal Chemistry* [online]. 2015, **58**(14), 5501-5521 [cit. 2016-07-17]. DOI: 10.1021/acs.jmedchem.5b00489. ISSN 0022-2623. Available from: http://pubs.acs.org/doi/abs/10.1021/acs.jmedchem.5b00489

[34] ZIDAR, Nace, Helena MACUT, Tihomir TOMAŠIČ, et al. N -Phenyl-4,5-dibromopyrrolamides and N -Phenylindolamides as ATP Competitive DNA Gyrase B Inhibitors: Design, Synthesis, and Evaluation. *Journal of Medicinal Chemistry* [online]. 2015, **58**(15), 6179-6194 [cit. 2016-07-17]. DOI: 10.1021/acs.jmedchem.5b00775. ISSN 0022-2623. Available from: http://pubs.acs.org/doi/abs/10.1021/acs.jmedchem.5b00775

[35] MCMURRY, John. *Organická chemie*. V Brně: VUTIUM, 2007. Překlady vysokoškolských učebnic. ISBN 978-80-214-3291-8.

[36] HANDY, Scott T., Jesse J. SABATINI, Yanan ZHANG a Inessa VULFOVA. Protection of poorly nucleophilic pyrroles. *Tetrahedron Letters*[online]. 2004, **45**(26), 5057-5060

[cit. 2016-08-02]. DOI: 10.1016/j.tetlet.2004.04.178. ISSN 00404039. Dostupné z: http://linkinghub.elsevier.com/retrieve/pii/S0040403904010147

[37] MOLINS-PUJOL, Antoni M. et al. Synthesis and nucleophilic properties of 1,2 -dialkyl-3-nitropyrroles and 1,5-dialkyl-4-nitropyrrole-2-carboxylic acid derivatives. *Journal of Chemical Society-Perkin Transactions 1*, 1996, **18**, 2277-2289.

[38] KHALAF, Abedawn I. et al. Distamycin Analogues with Enhanced Lipophilicity: Synthesis and Antimicrobial Activity. *Journal of Medicinal Chemistry*, 2004, **47**, 2133-2156. ISSN 0022-2623.

[39] MOTEKAITIS, R. J. et al. 3,4-Dihalopyrroles. *Journal of Organic Chemistry*, 1970, **35**, 2504-2511. DOI: 10.1021/jo00833a008. ISSN: 1520-6904.